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GELS FOR ENCAPSULATION OF BIOLOGICAL MATERIALS

This application is a Continuation of 08/510,089, filed August 1, 1995, which is a CIP of 07/958,870, filed 10/7/92, which is a CIP of 07/870,540 (abandoned), filed 4/20/92; this is also a CIP of 08/379,848, filed 1/27/95, which is a continuation of 08/022,687 filed 3/1/93 and issued as U.S.P.N. 5,410,016, which is a CIP of 07/843,485, filed 2/28/92 (abandoned); this is also a CIP of 08/336,393, filed 11/10/94, which is a continuation of 07/598,880, filed 10/15/90 (abandoned.)

BACKGROUND

Microencapsulation technology holds promise in many areas of medicine. For example, some important applications are treatment of diabetes (Goosen, M. F. A., et al. (1985) Biotechnology and Bioengineering, 27:146.), production of biologically important chemicals (Omata, T., et al. (1979) European J. Appl. Microbiol. Biotechnol., 6:207:215), evaluation of anti-human immuno-deficiency virus drugs (McMahon, J., et al., (1990) J. Nat. Cancer Inst., 82(22) 1761-1765), encapsulation of hemoglobin for red blood cell substitutes, and controlled release of drugs. During encapsulation using prior methods, cells are often exposed to processing conditions which are potentially cytotoxic. These conditions include heat, organic solvents and

non-physiological pH which can kill or functionally impair cells. Proteins are often exposed to conditions which are potentially denaturing and can result in loss of biological activity.

Further, even if cells survive processing conditions, the stringent requirements of encapsulating polymers for biocompatibility, chemical stability, immunoprotection and resistance to cellular overgrowth, restrict the applicability of prior art methods. For example, the encapsulating method based on ionic crosslinking of alginate (a polyanion) with polylysine or polyornithine (polycation) (Goosen, M. F. A., et al. (1985) Biotechnology and Bioengineering, 27:146) offers relatively mild encapsulating conditions, but the long-term mechanical and chemical stability of such ionically crosslinked polymers remains doubtful. Moreover, these polymers when implanted in vivo, are susceptible to cellular overgrowth (McMahon, J., et al. (1990) J. Nat. Cancer Inst., 82(22) 1761-1765) which restricts the permeability of the microcapsule to nutrients, metabolites, and transport proteins from the surroundings. This has been seen to possibly lead to starvation and death of encapsulated islets of Langerhans cells (O'Shea, G. M. et al. (1986) Diabetes, 35:943-946).

Thus, there is a need for a relatively mild cell encapsulation method which offers control over properties of the encapsulating polymer. The membranes must be non-toxically produced in the presence of cells, with the qualities of being

permselective, chemically stable, and very highly biocompatible. A similar need exists for the encapsulation of biological materials other than cells and tissues.

Biocompatibility

Materials are considered biocompatible if the material elicits either a reduced specific humoral or cellular immune response or does not elicit a nonspecific foreign body response that prevents the material from performing the intended function, and if the material is not toxic upon ingestion or implantation. The material also must not elicit a specific reaction such as thrombosis if in contact with the blood.

Use of Gels in Biomaterials

Gels made of polymers which swell in water such as poly (HEMA), water-insoluble polyacrylates, and agarose, have been shown to be capable of encapsulating islet cells and other animal tissue (Iwata, H., et al. (1989) Diabetes, 38:224-225; Lambert, F. V., et al. (1984) Appl. Biochem. Biotech, 10:101-105). However, these gels have undesirable mechanical properties. Agarose forms a weak gel, and the polyacrylates must be precipitated from organic solvents, thus increasing the potential for cytotoxicity. Dupuy et al. ((1988) J. Biomed. Mater. Res., 22:1061-1070) have reported the microencapsulation of islets by polymerization of acrylamide to form polyacrylamide gels. However, the polymerization process, if allowed to proceed rapidly to completion, generates local heat and requires the

presence of toxic cross-linkers. This usually results in mechanically weak gels whose immunoprotective ability has not been established. Moreover, the presence of a low molecular weight monomer is required which itself is cytotoxic.

Microcapsules formed by the coacervation of alginate and poly(L-lysine) have been shown to be immunoprotective e.g., O'Shea, G. M. et al. (1986) Diabetes, 35:943-946. However, implantation for periods up to a week has resulted in severe fibrous overgrowth on these microcapsules (McMahon, J., et al. (1990) J. Nat. Cancer Inst., 82(22) 1761-1765; O'Shea, G. M. et al. (1986) Diabetes, 35:943-946).

Synthetic Biodegradable Polymers

The field of biodegradable polymers has developed rapidly since the synthesis and biodegradability of polylactic acid was first reported by Kulkarni et al., (1966) Arch. Surg., 93:839. Several other polymers are known to biodegrade, including polyanhydrides and polyorthoesters, which take advantage of labile backbone linkages, as reported by Domb et al., 1989 Macromolecules, 22:3200; Heller et al., 1990 BIODEGRADABLE POLYMERS AS DRUG DELIVERY SYSTEMS, Chasin, M. and Langer, R., Eds., Dekker, New York, 121-161. Since it is desirable to have polymers that degrade into naturally occurring materials, polyaminoacids have been synthesized, as reported by Miyake et al., (1974), for *in vivo* use. This was the basis for using polyesters (Holland et al., 1986 Controlled Release, 4:155-

180) of α-hydroxy acids (*viz.*, lactic acid, glycolic acid), which remain the most widely used biodegradable materials for applications ranging from closure devices (sutures and staples) to drug delivery systems (U.S. Patent No. 4,741,337 to Smith et al.; Spilizewski et al., (1985) J. Control. Rel. 2:197-203).

The time required for a polymer to degrade can be tailored by selecting appropriate monomers. Differences in crystallinity also alter degradation rates. Due to the relatively hydrophobic nature of these polymers, actual mass loss only begins when the oligomeric fragments are small enough to be water soluble. Hence, initial polymer molecular weight influences the degradation rate.

Degradable polymers containing water-soluble polymer elements have been described. Sawhney et al., (1990) J. Biomed. Mater. Res. 24:1397-1411, copolymerized lactide, glycolide and ε-caprolactone with PEG to increase its hydrophilicity and degradation rate. U.S. Patent No. 4,716,203 to Casey et al. (1987) synthesized a PGA-PEG-PGA block copolymer, with PEG content ranging from 5-25% by mass. U.S. Patent No. 4,716,203 to Casey et al. (1987) also reports synthesis of PGA-PEG diblock copolymers, again with PEG ranging from 5-25%. U.S. Patent No. 4,526,938 to Churchill et al. (1985) described noncrosslinked materials with MW in excess of 5,000, based on similar compositions with PEG; although these materials are not water soluble. Cohn et al. (1988) J. Biomed. Mater. Res. 22:993-1009

described PLA-PEG copolymers that swell in water up to 60%; these polymers also are not soluble in water, and are not crosslinked. These materials all use both water-soluble polymers and degradable polymers, and they are all insoluble in water, collectively swelling up to about 60%.

Degradable materials of biological origin are well known, for example, crosslinked gelatin. Hyaluronic acid has been crosslinked and used as a degradable swelling polymer for biomedical applications (U.S. Patent No. 4,987,744 to Della Valle et al., U.S. Patent 4,957,744 to Della Valle et al. (1991) Polym. Mater. Sci. Eng., 62:731-735).

Use of Biodegradable Materials for Controlled Drug Release

Most hydrophilic drugs are mechanically dispersed as suspensions within solutions of biodegradable polymers in organic solvents. Protein and enzyme molecular conformations are frequently different under these circumstances than they would be in aqueous media. An enzyme dispersed in such a hydrophobic matrix is usually present in an inactive conformation until it is released into the surrounding aqueous environment subsequent to polymer degradation. Additionally, some proteins may be irreversibly denatured by contact with organic solvents used in dispersing the protein within the polymer.

Use of PEO in Biomaterials

The use of poly(ethylene oxide) (PEO) to increase biocompatibility is well documented in the literature. The

presence of grafted PEO on the surface of bovine serum albumin has been shown by Abuchowski, A. et al. ((1977) J. Biol. Chem., 252:3578) to reduce immunogenicity in a rabbit and to increase circulation times of exogenous proteins in animals. The biocompatibility of algin-poly(L-lysine) microcapsules has been significantly enhanced by incorporating a graft copolymer of PLL and PEO on the microcapsule surface (Sawhney, et al., Biomaterials 13:863-870 (1991)).

The grafting of methoxy PEO onto polyacrylonitrile surfaces was seen by Miyama et al. ((1988) J. Appl. Polym. Sci., 35:115-125) to render the polyacrylonitrile surface relatively non-thrombogenic. Nagoaka et al. (Polymers as Biomaterials, Shalaby, S. W. ed., Plenum Press, New York) synthesized a graft copolymer of methacrylates with PEO and found the resulting polymer to be highly non-thrombogenic. Desai and Hubbell have immobilized PEO on poly(ethylene terepthalate) surfaces by forming a physical interpenetrating network (Desai et al., (1992) Macromolecules 25:226); they have shown these surfaces to be highly resistant to thrombosis (Desai et al, (1991) Biomaterials, 12:144) and to both mammalian and bacterial cell growth (Desai, et al., submitted).

PEO is a unique polymer in terms of structure. The PEO chain is highly water soluble and highly flexible. PEO chains have an extremely high motility in water and are essentially non-ionic in structure. The synthesis and characterization of PEO

derivatives which can be used for attachment of PEO to various surfaces, proteins, drugs etc. has been reviewed (Harris, (1985) JNS-Rev. Macromol. Chem. Phys., C25:325-373). Other polymers are also water soluble and non-ionic, such as poly(N-vinyl pyrrolidinone) and poly(ethyl oxazoline). These have been used to reduce interaction of cells with tissues. Desai et al. (1991) Biomaterials, 12:144. Water soluble ionic polymers, such as hyaluronic acid, have also been used to reduce cell adhesion to surfaces and can similarly be used.

Electron beam cross-linking has been used to synthesize PEO hydrogels, and these biomaterials have been reported to be non-thrombogenic (Sun, et al., (1987) Polymer Prepr., 28:292-294; Dennison, H.A., (1986) Ph.D. Thesis. Massachusetts Institute of Technology). However, use of an electron beam precludes the presence of any living tissue due to the sterilizing effect of this radiation. Also, the networks produced are difficult to characterize due to the non-specific cross-linking induced by the electron beam.

Photopolymerizable PEG diacrylates have been used to entrap yeast cells for fermentation and chemical conversion (Kimura et al. (1981) Eur. J. Appl. Microbio. Biotechnol., 11:78-80; Omata et al., (1981) Eur. J. Appl. Microbial Biotechnol., 11:199-204; Okada et al. (1987) Appl. Microbiol. Biotechnol., 26:112-116). Other methods for encapsulation of cells within materials photopolymerizable with short wavelength ultraviolet

radiation have been used with microbial cells (Tanaka, et al., (1977) Eur. J. Biochem, 80:193-197; Omata, et al., (1979) European J. Appl. Microbiol. Biotechnol., 6:207-215; Omata, et al., (1979) Eur. J. Appl. Microhiol. Biotechnol. 8:143-155; Chun, et al., (1981)J. Gen. App. Microbiol., 27:505-509; Fukui, et al., (1976) Febs Letters, 66:2; Fukui, et al., (1984) Advances in Biochemical Engineering and Biotechnology, 29:1-33). However, yeast cells and some microbial cells are much hardier and resistant to adverse environments, elevated temperatures, and short wavelength ultraviolet radiation than mammalian cells and human tissues.

There are several problems with these methods, including the use of methods and/or materials which are thrombogenic or unstable *in vivo*, or require polymerization conditions which tend to destroy living mammalian tissue or biologically active molecules, for example, short wavelength ultraviolet radiation. In order to encapsulate living tissue for implantation in a human or other mammalian subject, the polymerization conditions must not destroy the living tissue, and the resulting polymer-coated cells must be biocompatible.

There is also a need to encapsulate materials within a very thin layer of material that is permeable to nutrients and gases, yet strong and non-immunogenic. For example, for transplantation of islets of Langerhans, the islets, which have a diameter of 100 to 200 microns, have in the past been

encapsulated within microspheres that have a diameter of 400 to 1000 microns. This large diameter can result in slowed diffusion of nutritional molecules and large transplantation volumes.

In summary, there is a need for materials, and methods of use thereof, which can be used to encapsulate cells and tissues or biologically active molecules which are biocompatible, do not elicit specific or non-specific immune responses, and which can be polymerized in contact with living cells or tissue without injuring or killing the cells, within a very short time frame, and in a very thin layer. An important aspect of the use of these materials *in vivo* is that they must be polymerizable within the time of a short surgical procedure or before the material to be encapsulated disperses, is damaged or dies.

It is therefore an object of the present invention to provide a polymeric material that can be polymerized in contact with living cells and tissues, and in a very short time period.

It is a further object of the present invention to provide a polymeric material which is biocompatible and resistant to degradation for a specific time period.

It is a still further object of the present invention to provide a polymeric material which is permeable to nutrients and gases yet can protect cells and tissues from *in vivo* attack by other cells.

It is yet a further object of the present invention to provide implantable biodegradable materials and biodegradable materials for encapsulation of cells and tissue.

It is another object of the present invention to provide biogels which can be both ionically and covalently crosslinked.

It is an additional object of the present invention to provide a crosslinked biocompatible material which has at least one ionically crosslinked component and at least one covalently crosslinked component.

SUMMARY OF THE INVENTION

This invention provides novel methods for the formation of biocompatible membranes around biological materials using photopolymerization of water soluble molecules. The membranes can be used as a covering to encapsulate biological materials or biomedical devices, as a "glue" to cause more than one biological substance to adhere together, or as carriers for biologically active species.

Several methods for forming these membranes are provided. Each of these methods utilizes a polymerization system containing water-soluble macromers, species which are at once polymers and macromolecules capable of further polymerization. The macromers are polymerized using a photoinitiator (such as a dye), optionally a cocatalyst, optionally an accelerator, and

radiation in the form of visible or long wavelength UV light. The reaction occurs either by suspension polymerization or by interfacial polymerization. The polymer membrane can be formed directly on the surface of the biological material, or it can be formed on material which is already encapsulated.

The macromers, which are water soluble or substantially water soluble, are too large to diffuse into the cells to be coated. Examples of macromers include highly biocompatible PEG hydrogels, which can be rapidly formed in the presence or absence of oxygen, without use of toxic polymerization initiators, at room or physiological temperatures, and at physiological pH.

Some macromers of this invention include at least one water soluble region, at least one region which is biodegradable, usually by hydrolysis, and at least two free radical-polymerizable regions. The regions can, in some embodiments, be both water soluble and biodegradable.

Ultrathin membranes can be formed by the methods described herein. These ultrathin membranes allow for optimal diffusion of nutrient and bioregulator molecules across the membrane, and great flexibility in the shape of the membrane. Such thin membranes produce encapsulated material with optimal economy of volume. Biological material thus coated can be packed into a relatively small space without interference from bulky membranes.

The thickness and pore size of membranes formed can be varied. This variability allows for "perm-selectivity" -- membranes can be adjusted to the desired degree of porosity, allowing only preferred molecules to permeate the membrane, while acting as a barrier against larger undesired molecules. Thus, the membranes are immunoprotective in that they prevent the transfer of antibodies or cells of the immune system.

When the encapsulated biological material is cellular in nature, the absence of small monomers in the polymerization solution prevents the diffusion of toxic molecules into the cell. In this manner the present invention provides a polymerization system which is more biocompatible than any available in the prior art.

Additionally, the polymerization method can utilize short bursts of visible or long wavelength UV light which is nontoxic to biological material. Bioincompatible polymerization initiators employed in the prior art are also eliminated.

According to the present invention, membrane formation occurs under physiological conditions. Thus, no damage is done to the enclosed biological material due to harsh pH, temperature, or ionic conditions.

Because the membrane adheres to the biological material, the membrane can be used as an adhesive to fasten more than one biological substance together. The macromers are polymerized in the presence of these substances which are in close proximity.

The membrane forms in the interstices, effectively gluing the substances together.

Additionally, utilizing the tendency of the membrane to adhere to biological material, a membrane can be formed around or on a biologically active substance to act as a carrier for that substance.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows schematically illustrated macromers of the present invention where _____ is a water soluble core such as PEG; ~~~~~ is a hydrolyzably degradable extension such as a polyglycolide; ====== is a polymerizable end cap or side chain such as an acrylate; and ------ is a water-soluble and hydrolyzable portion such as a hyaluronate.

Figure 2A is a schematic of dye-initiated polymerization of a PEG layer around crosslinked alginate microspheres.

Figure 2B is a photomicrograph of the alginate/poly(L-lysine) microspheres containing human islets of Langerhans coated with a PEG 18.5K tetraacrylate hydrogel using the dye binding method depicted in Figure 2A.

Figure 3 is a schematic of photopolymerization of a PEG coating on alginate-poly(L-lysine) microspheres suspended in mineral oil.

Figure 4 is a photomicrograph of Islets of Langerhans isolated from a human pancreas encapsulated in a PEG 18.5K tetraacrylate hydrogel.

Figure 5 is a schematic representation of coextrusion apparatus used for microencapsulation using laser polymerization

Figure 6 is a photomicrograph of microspheres produced by laser polymerization of PEG 400 diacrylate around cells.

Figure 7A is a photomicrograph of alginate-PLL microspheres recovered after 4 days following implantation i.p. in mice.

Figure 7B is a photomicrograph of Alginate-PLL microspheres coated with a PEG 18.5K Da tetraacrylate, using the dye diffusion method depicted in Figure 1.

Figures 8A-F is a graph of the number of cells versus gel composition, for the unattached cells obtained from lavage of the peritoneal cavity in mice with different PEO overcoat gel compositions: a - 18.5k; b - 10% 0.5k, 90% 18.5k; c - 50% 18.5k, 50% 0.4k; d - 10% 0.4k, 90% 35k; e - 50% 0.4k, 50% 35k; and f - alginate-poly(L-lysine) control.

Figure 9 is a graph of the % protein released versus time in minutes, for diffusion of bovine serum albumin (open squares), human IgG (triangles) and human fibrinogen (closed squares) through a PEO 18.5K-tetraacrylate gel.

Figure 10 is a graph of the % diffusion of bovine serum albumin over time in minutes through PEO 400 diacrylate (open squares) and PEG 18.5K-tetracrylate (triangles) gels.

Figure 11A is a graph of the length in mm of gel produced by argon ion laser induced polymerization versus log (time) (ms) of trimethylolpropane using an amine and ethyl eosin initiation system.

Figure 11B is a photomicrograph of the spikes formed as a result of laser irradiation of ethoxylated trimethylol propane triacrylate for durations of 67 ms, 125 ms, 250 ms, 500 ms, and 1 sec.

Figure 12A is a photomicrograph of human foreskin fibroblasts cultured for 6 h on a glass coverslip coated with PEG 18.5K-tetraacrylate gel.

Figure 12B is a photomicrograph of human foreskin fibroblasts cultured for 6 h on a glass that was not coated with PEG.

Figure 13 is a photomicrograph of PEG 18.5K-tetraacrylate microspherical gels, implanted in mice, and explanted after 4 days, showing very little fibrous overgrowth.

Figures 14A-C are creep curves for PEG diacrylate and tetraacrylate gels; test and recovery loads are given below the abscissa: A - 1k; B - 6K; and C - 18.5K PEG gels.

Figure 15 shows the degree of photopolymerization (dp) calculated and found by NMR.

Figure 16A shows Human foreskin fibroblasts cultured for six hours on glass coverslips coated with PEG 18.5K-glycolide diacrylate (18.5KG).

Figure 16B shows Human foreskin fibroblasts cultured for six hours on glass coverslips not coated with PEG.

Figure 17A shows the release of BSA from a PEG 1K (1000 molecular weight PEG) glycolide diacrylate with glycolide extensions (1 KG) hydrogel into PBS.

Figure 17B shows release of lysozyme from PEG 18.5K-DL-lactide tretraacrylate (18.5KL) into PBS.

Figure 18A shows release of active recombinant tPA from a PEG 1K lactide diacrylate (1KL) hydrogel.

Figure 18B shows release of active recombinant t-PA from PEG 4K glycolide diacrylate (4KG) hydrogel.

Figure 18C shows release of active recombinant tPA from a PEG 18.5K-glycolide diacrylate (18.5KG) hydrogel into PBS.

Figure 19A is a superior view of rabbit uterine horn used as a control. Distorted horn anatomy with 66% adhesions is evident. The horns are folded upon themselves.

Figure 19B is a superior view of rabbit uterine horn treated with a photopolymerized biodegradable hydrogel, PEG 18.5KL. Horn anatomy is normal, with no adhesion bands visible.

Figure 20A is an environmental scanning electron micrograph (ESEM) of an untreated blood vessel following trauma.

Figure 20B is an ESEM of a polymer coated blood vessel following trauma.

DETAILED DESCRIPTION

By a variety of methods, this invention provides a means for creating biocompatible membranes of varying thickness on the surface of a variety of biological materials. The polymerization occurs by a free-radical reaction, causing a "macromer" with at least two ethylenically unsaturated moieties to form a crosslinked polymer. The components of this reaction are:

(1) a photoinitiator, preferably eosin dye;

(2) a "macromer," preferably polyethylene glycol (PEG) diacrylate, m.w. 18.5 kD. This component is at once a polymer and a macromolecule capable of further polymerization;

(3) optionally a cocatalyst, preferably triethanolamine; and

(4) optionally, an accelerator.

These components are mixed in varying combinations, and the mixture is exposed to longwave UV or visible light ("radiation"), preferably of wavelength 350-700 nm, most preferred at 365-514 nm, to initiate polymerization. A network is formed as the macromers polymerize in a variety of directions.

POLYMERIZATION

Four methods are used to effect polymerization to form biocompatible membranes. These are referred to below as the "bulk suspension polymerization" method, the "microcapsule suspension polymerization" method, the "microcapsule interfacial polymerization" method, and the "direct interfacial polymerization" method. They utilize either suspension or interfacial polymerization techniques on either coated or uncoated biological material.

1. BULK SUSPENSION POLYMERIZATION METHOD

In this embodiment of the invention the core biological material is mixed in an aqueous macromer solution (composed of the macromer, cocatalyst and optionally an accelerator) with the photoinitiator. Small globular geometric structures such as spheres, ovoids, or oblongs are formed, preferably either by coextrusion of the aqueous solution with air or with a non-miscible substance such as oil, preferably mineral oil, or by agitation of the aqueous phase in contact with a non-miscible phase such as an oil phase to form small droplets. The macromer in the globules is then polymerized when exposed to radiation. Because the macromer and initiator are confined to the globules, the structure resulting from polymerization is a capsule in which the biological material is enclosed. This is a "suspension polymerization" whereby the entire aqueous portion of the globule

polymerizes to form a thick membrane around the cellular material.

2. MICROCAPSULE SUSPENSION POLYMERIZATION METHOD

This embodiment of the invention employs microencapsulated material as a core about which the macromer is polymerized in a suspension polymerization reaction. The biological material is first encapsulated, such as in an alginate microcapsules. The microcapsule is then mixed as in the first embodiment with the macromer solution and the photoinitiator, and then polymerized by radiation. In the event an ionically crosslinkable material such as alginate is used for the first encapsulation, this results in a biocompatible material which is both ionically crosslinked and covalently crosslinked.

This method takes advantage of the extreme hydrophilicity of PEG macromer, and is especially suited for use with hydrogel microcapsules such as alginate-poly(L-lysine). The microsphere is swollen in water. When a macromer solution (with the initiating system) is forced to phase separate in a hydrophobic medium, such as mineral oil, the PEG macromer solution prefers to stay on the hydrophilic surface of the alginate microcapsule. When this suspension is irradiated, the PEG macromer undergoes polymerization and gelation, forming a thin layer of polymeric, water insoluble gel around the microsphere. Agarose beads have been used in an analogous way by Gin et al.(1987) J. Microencapsulation, 4:239-242 as scaffolds to

carry out polymerization of acrylamide. However, that method is limited by potential toxicity associated with the use of a low molecular weight monomer, as opposed to the macromeric precursors of the present invention.

This technique preferably involves coextrusion of the microcapsule in a solution of macromer and photoinitiator, the solution being in contact with air or a liquid which is non-miscible with water, to form droplets which fall to a container such as a petri dish containing a solution such as mineral oil in which the droplets are not miscible. The non-miscible liquid is chosen for its ability to maintain droplet formation. Additionally, if the membrane-encapsulated material is to be injected or implanted in an animal, any residue should be non-toxic and non-immunogenic. Mineral oil is a preferred non-miscible liquid.

On the petri dish the droplets are exposed to radiation which causes polymerization. This coextrusion technique results in a crosslinked polymer coat of greater than 50 microns thickness. Alternatively, the microcapsules may be suspended in a solution of macromer and photoinitiator which is agitated in contact with a non-miscible phase such as an oil phase. The emulsion which results is irradiated to form a polymer coat, again of greater than 50 microns thickness.

3. <u>MICROCAPSULE INTERFACIAL POLYMERIZATION METHOD</u>

In this embodiment, the biological material is also microencapsulated as in the previous method. However, rather than suspension polymerization, interfacial polymerization is utilized to form the membrane. This involves coating the microcapsule with photoinitiator, suspending the microcapsule in the macromer solution, and immediately irradiating. By this technique a thin polymer coat, of less than 50 microns thickness, is formed about the microcapsule, because the photoinitiator is present only at the microcapsule surface and is given insufficient time to diffuse far into the macromer solution. As a result, the initiator is present in only a thin shell of the aqueous solution, causing a thin layer to be polymerized.

When the microcapsules are in contact with dye solution, the dye penetrates into the inner core of the microcapsule as well as adsorbing to the surface. When such a microcapsule is put into a solution containing a macromer and, optionally, a cocatalyst such as triethanolamine, and exposed to laser light, initially all the essential components of the reaction are present only at and just inside the interface of microcapsule and macromer solution. Hence, the polymerization and gelation (if multifunctional macromer is used) initially takes place only at the interface, just beneath it, and just beyond it. If left for longer periods of time, the dye starts

diffusing from the inner core of the microsphere into the solution; similarly, macromers start diffusing inside the core.

Polymerization and subsequent gelation are very rapid (typical gelation times are 100 ms) (Fouassier, et al., (1985) J. Polym. Sci., Polym. Chem. Ed., 23:569; Chesneau, et al., (1985) Die Ange. Makromol. Chemie, 135:41, (1988) Makromol. Chem., Rapid Commun., 9:223). Because diffusion is a much slower process than polymerization, not the entire macromer solution is polymerized or gelled. Essentially the reaction is restricted to the near surface only. The dye, being a smaller molecule and being weakly bound to the capsule materials, keeps diffusing out of the microsphere. If this diffusion occurs under laser irradiation, then dye at the interface is used continuously to form a thicker gel layer. The thickness of the coating can thus be directed by controlling the reaction conditions.

A schematic representation of this process is shown in Figure 2A. The amount, thickness or size and rigidity of the gel formed will depend on the size and intensity of the beam, time of exposure, initiator, macromer molecular weight, and macromer concentration (see below). Alginate/PLL microspheres containing islets coated by this technique are shown in Figure 2B.

4. DIRECT INTERFACIAL POLYMERIZATION METHOD

The fourth embodiment of this invention utilizes interfacial polymerization to form a membrane directly on the surface of the biological material. This results in the smallest

capsules and thus achieves optimal economy of volume. Tissue is directly coated with photoinitiator, emersed in the macromer solution, and immediately irradiated. This technique results in a thin polymer coat surrounding the tissue since there is no space taken up by a microcapsule, and the photoinitiator is again present only in a thin shell of the macromer solution.

USE AS AN ADHESIVE

It is usually difficult to get good adhesion between polymers of greatly different physicochemical properties. The concept of a surface physical interpenetrating network was presented by Desai and Hubbel (Desai et al. (1992) Macromolecules 25:226). This approach to incorporating into the surface of one polymer a complete coating of a polymer of considerably different properties involved swelling the surface of the polymer to be modified (base polymer) in a mutual solvent, or a swelling solvent, for the base polymer and for the polymer to be incorporated (penetrant polymer). The penetrant polymer diffused into the surface of the base polymer. This interface was stabilized by rapidly precipitating or deswelling the surface by placing the base polymer in a nonsolvent bath. This resulted in entanglement of the penetrant polymer within the matrix of the base polymer at its surface in a structure that was called a surface physical interpenetrating network.

This approach can be improved upon by photopolymerizing the penetrant polymer upon the surface of the base polymer in the swollen state. This results in much enhanced stability over that of the previous approach and in the enhancement of biological responses to these materials. The penetrant may be chemically modified to be a prepolymer (macromer), i.e. capable of being polymerized itself. This polymerization can be initiated thermally or by exposure to visible, ultraviolet, infrared, gamma ray, or electron beam irradiation, or to plasma conditions. In the case of the relatively nonspecific gamma ray or electron beam radiation reaction, chemical incorporation of particularly reactive sites may not be necessary.

Polyethylene glycol (PEG) is a particularly useful penetrant polymer for biomedical applications where the lack of cell adhesion is desired. The previous work had demonstrated an optimal performance at a molecular weight of 18,500 D without chemical crosslinking. PEG prepolymers can be readily formed by acrylation of the hydroxyl groups at its termini or elsewhere within the chain. These prepolymers can be readily polymerized by the above described radiation methods. Photoinititated polymerization of these prepolymers is particularly convenient and rapid. There are a variety of visible light initiated and ultraviolet light initiated reactions that are initiated by light absorption by specific photochemically reactive dyes, described elsewhere herein. This same approach can be used for biomedical

purposes with other water-soluble polymers, such as poly(N-vinyl pyrrolidinone), poly(N-isopropyl acrylamide), poly(ethyl oxazoline) and many others.

Additionally, it is usually difficult to obtain adhesives for wet surfaces and tissues. Water soluble prepolymers, for example PEG diacrylates, can be used for this purpose. When a water soluble polymer is placed in aqueous solution upon a tissue, the polymer diffuses into the surface of the tissue, within the protein and polysaccharide matrix upon the tissue but not within the cells themselves. When the water soluble polymer is a prepolymer and a visible, ultraviolet or infrared photoinitiator is included, the polymer penetrant may be exposed to the appropriate light to gel the polymer. In this way, the polymer is crosslinked within and around the matrix of the tissue in what is called an interpenetrating network. If the prepolymer is placed in contact with two tissues and the prepolymer is illuminated, then these two tissues are adhered together by the intermediate polymer gel.

BIOLOGICAL MATERIALS

Due to the biocompatibility of the materials and techniques involved, a wide variety of materials can be used in conjunction with the present invention. For encapsulation, the techniques can be used with mammalian tissue and/or cells, as well as sub-cellular organelles and other isolated sub-cellular

components. The membranes can be crafted to meet the perm-selectivity needs of the biological material enclosed. Cells which are to be used to produce desired products such as proteins are optimally encapsulated by this invention.

Examples of cells which can be encapsulated are primary cultures as well as established cell lines, including transformed cells. These include but are not limited to pancreatic islet cells, human foreskin fibroblasts, Chinese hamster ovary cells, beta cell insulomas, lymphoblastic leukemia cells, mouse 3T3 fibroblasts, dopamine secreting ventral mesencephalon cells, neuroblastoid cells, adrenal medulla cells, and T-cells. As can be seen from this partial list, cells of all types, including dermal, neural, blood, organ, muscle, glandular, reproductive, and immune system cells can be encapsulated successfully by this method. Additionally, proteins (such as hemoglobin), polysaccharides, oligonucleotides, enzymes (such as adenosine deaminase), enzyme systems, bacteria, microbes, vitamins, cofactors, blood clotting factors, drugs (such as TPA, streptokinase or heparin), antigens for immunization, hormones, and retroviruses for gene therapy can be encapsulated by these techniques.

The biological material can be first enclosed in a structure such as a polysaccharide gel. (Lim, U.S.P.N. 4,352,883; Lim, U.S.P.N. 4,391909; Lim, U.S.P.N. 4,409,331; Tsang, et al., U.S.P.N. 4,663,286; Goosen et al., U.S.P.N.

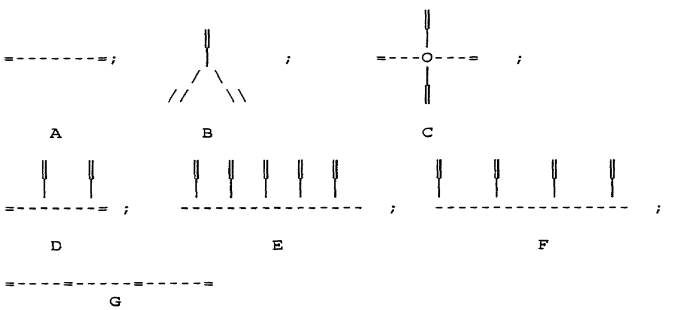
4,673,556; Goosen et al., U.S.P.N. 4,689,293; Goosen et al., U.S.P.N. 4,806,355; Rha et al., U.S.P.N. 4,744,933; Rha et al., U.S.P.N. 4,749,620, incorporated herein by reference.) Such gels can provide additional structural protection to the material, as well as a secondary level of perm-selectivity. If alginate is used, it is preferred that the alginate be relatively high in α-L-guluronic acid content. This "high G" content increases the biocompatibility of the material. The alginate should be at least 60% α-L-guluronic acid, and more preferably at least 70% α-L-guluronic acid.

MACROMERS

Polymerization via this invention utilizes macromers rather than monomers as the building blocks. The macromers are small polymers which are susceptible to polymerization into the larger polymer membranes of this invention. Polymerization is enabled because the macromers contain sites of unsaturation, e.g., carbon-carbon double bond moieties, carbon-carbon triple bond moieties, and the like, as well as sites of unsaturation between carbon atoms and heteroatoms and between two heteroatoms. Examples of carbon-carbon double bonds useful in this invention include acrylate, methacrylate, ethacrylate, 2-phenyl acrylate, 2-chloro acrylate, 2-bromo acrylate, itaconate, acrylamide, methacrylamide, and styrene groups. The macromers are water

soluble compounds and are non-toxic to biological material before and after polymerization.

A wide variety of substantially water soluble polymers exist, some of which are illustrated schematically below. (_____) represents a substantially water soluble region of the polymer, and (=) represents a free radical polymerizable species. Examples include:



Examples of A include PEG diacrylate, from a PEG diol; of B include PEG triacrylate, formed from a PEG triol; of C include PEG-cyclodextrin tetraacrylate, formed by grafting PEG to a cyclodextrin central ring, and further acrylating; of D include PEG tetraacrylate, formed by grafting two PEG diols to a bis epoxide and further acrylating; of E include hyaluronic acid methacrylate, formed by acrylating many sites on a hyaluronic acid chain; of F include PEG-hyaluronic acid-multiacrylate,

formed by grafting PEG to hyaluronic acid and further acrylating; of G include PEG-unsaturated diacid ester formed by esterifying a PEG diol with an unsaturated diacid.

Polysaccharides include, for example, alginate (preferably high-G alginate), hyaluronic acid, chondroitin sulfate, dextran, dextran sulfate, heparin, heparin sulfate, heparan sulfate, chitosan, gellan gum, xanthan gum, guar gum, and K-carrageenan. Proteins, for example, include gelatin, collagen, elastin and albumin, whether produced from natural or recombinant sources. In the event that a substance such as alginate or hyaluronic acid is used, the resulting macromers will be both ionically crosslinkable and covalently crosslinkable.

Photopolymerizable substituents preferably include acrylates, diacrylates, oligoacrylates, dimethacrylates, or oligomethoacrylates, and other biologically acceptable photopolymerizable groups.

Synthetic Polymeric Macromers

The water-soluble macromer may be derived from water-soluble polymers including, but not limited to, poly(ethylene oxide) (PEO), poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP), poly(ethyloxazoline) (PEOX) polyaminoacids, pseudopolyamino acids, and polyethyloxazoline, as well as copolymers of these with each other or other water soluble polymers or water insoluble polymers, provided that the conjugate is water soluble. An example of a water soluble

conjugate is a block copolymer of polyethylene glycol and polypropylene oxide, commercially available as a Pluronic™ surfactant.

Polysaccharide Macromers

Polysaccharides such as alginate (preferably high-G alginate), hyaluronic acid, chondroitin sulfate, dextran, dextran sulfate, heparin, heparin sulfate, heparan sulfate, chitosan, gellan gum, xanthan gum, guar gum, water soluble cellulose derivatives, and carrageenan, which are linked by reaction with hydroxyls or amines on the polysaccharides can also be used to form the macromer solution. As noted above, in the event that alginate, hyaluronic acid, or other ionically gellable materials are used, the macromers are both ionically crosslinkable and covalently crosslinkable.

Protein Macromers

Proteins such as gelatin, collagen, elastin, zein, and albumin, whether produced from natural or recombinant sources, which are made free-radical polymerization by the addition of carbon-carbon double or triple bond-containing moieties, including acrylate, diacrylate, methacrylate, ethacrylate, 2-phenyl acrylate, 2-chloro acrylate, 2-bromo acrylate, itaconate, oliogoacrylate, dimethacrylate, oligomethacrylate, acrylamide, methacrylamide, styrene groups, and other biologically acceptable photopolymerizable groups, can also be used to form the macromer solution.

MACROMERS FOR BIODEGRADABLE GELS

In general terms, the macromers for biodegradable gels are polymers that are soluble in aqueous solutions, or nearly aqueous solutions, such as water with added dimethylsulfoxide. They have three components including a biodegradable region, preferably hydrolyzable under *in vivo* conditions, a water soluble region, and at least two polymerizable regions. Examples of these structures are shown in Figure 1.

Structure A in Figure 1 shows a macromer having a water soluble region (_____), a water soluble and degradable component (------) appended to one another. Each has a polymerizable end cap (======). Structure B shows a major water soluble component or core region (_____) extended at either end by a degradable or hydrolyzable component (~~~~~~) and terminated by, at either end, a polymerizable component (======). Structure C shows a central degradable or hydrolyzable component (~~~~~~) bound to a water soluble component (_____) capped at either end by a polymerizable component (======). Structure D shows a central water soluble component (_____) with numerous branches of hydrolyzable components (~~~~~~), each hydrolyzable component being capped with a polymerizable component (======). Structure E shows a central biodegradable, hydrolyzable component (~~~~~~) with three water soluble branches (_____), each water soluble branch being capped by a polymerizable component (======). Structure F shows a long central water soluble and hydrolyzable

component (------), each end being capped by a polymerizable component (======). Structure G shows a central water soluble and hydrolyzable component (------) capped at both ends by a hydrolyzable component (~~~~~~), each hydrolyzable component being capped by a polymerizable component (======). Structure H shows a central water soluble and degradable or hydrolyzable component (------) with end caps or branches of a polymerizable component (======). Structure I shows a central water soluble component (______) in circular form with water soluble branches extended by a hydrolyzable component (~~~~~~) capped by a polymerizable component (======). Lastly, Structure J in Figure 1 shows a circular water soluble core component (______) with degradable branches (~~~~~~), each being capped by a polymerizable component (~~~~~~).

The various structures shown in Figure 1 are exemplary only. Those skilled in the art will understand many other possible combinations which could be utilized for the purposes of the present invention.

Used herein is the term "at least substantially water soluble." This is indicative that the solubility should be at least about 1 g/100 ml of aqueous solution or in aqueous solution containing small amounts of organic solvent, such as dimethylsulfoxide. By the term "polymerizable" is meant that the regions have the capacity to form additional covalent bonds resulting in macromer interlinking, for example, carbon-carbon

double bonds of acrylate-type molecules. Such polymerization is characteristically initiated by free-radical formation, for example, resulting from photon absorption of certain dyes and chemical compounds to ultimately produce free-radicals.

In a preferred embodiment, a hydrogel begins with a biodegradable, polymerizable, macromer including a core, an extension on each end of the core, and an end cap on each extension. The core is a hydrophilic polymer or oligomer; each extension is a biodegradable polymer or oligomer; and each end cap is an oligomer, dimer or monomer capable of cross-linking the macromers. In a particularly preferred embodiment, the core includes hydrophilic poly(ethylene glycol) oligomers of molecular weight between about 400 and 30,000 Da; each extension includes biodegradable poly (α-hydroxy acid) oligomers of molecular weight between about 200 and 1200 Da; and each end cap includes an acrylate-type monomer or oligomer (i.e., containing carbon-carbon double bonds) of molecular weight between about 50 and 200 Da which are capable of cross-linking and polymerization between copolymers. More specifically, a preferred embodiment incorporates a core consisting of poly(ethylene glycol) oligomers of molecular weight between about 8,000 and 10,000 Da; extensions consisting of poly(lactic acid) oligomers of molecular weight about 250 Da; and end caps consisting acrylate moieties of about 100 Da molecular weight.

Those skilled in the art will recognize that oligomers of the core, extensions and end caps may have uniform compositions or may be combinations of relatively short chains or individual species which confer specifically desired properties on the final hydrogel while retaining the specified overall characteristics of each section of the macromer. The lengths of oligomers referred to herein may vary from two mers to many, the term being used to distinguish subsections or components of the macromer from the complete entity.

Water Soluble Regions

In preferred embodiments, the core water soluble region can consist of poly(ethylene glycol), poly(ethylene oxide), poly(vinyl alcohol), poly(vinylpyrrolidone), poly(ethyloxazoline), poly(ethylene oxide)-co-poly(propyleneoxide) block copolymers, polysaccharides or carbohydrates such as hyaluronic acid, dextran, heparan sulfate, chondroitin sulfate, heparin, or alginate (preferably high-G alginate), proteins such as gelatin, collagen, albumin, ovalbumin, or polyamino acids.

Biodegradable Regions

The biodegradable region is preferably hydrolyzable under *in vivo* conditions. For example, hydrolyzable group may be polymers and oligomers of glycolide, lactide, ε-caprolactone, other hydroxy acids, and other biologically degradable polymers that yield materials that are non-toxic or present as normal

metabolites in the body. Preferred poly(α-hydroxy acid)s are poly(glycolic acid), poly(DL-lactic acid) and poly(L-lactic acid). Other useful materials include poly(amino acids), poly(anhydrides), poly(orthoesters), poly(phosphazines) and poly(phosphoesters). Polylactones such as poly(ϵ-caprolactone), poly(ϵ-caprolactone), poly(δ-valerolactone) and poly(gamma-butyrolactone), for example, are also useful. The biodegradable regions may have a degree of polymerization ranging from one up to values that would yield a product that was not substantially water soluble. Thus, monomeric, dimeric, trimeric, oligomeric, and polymeric regions may be used.

Biodegradable regions can be constructed from polymers or monomers using linkages susceptible to biodegradation, such as ester, peptide, anhydride, orthoester, phosphazine and phosphoester bonds.

<u>Polymerizable Regions</u>

The polymerizable regions are preferably polymerizable by photoinitiation by free radical generation, most preferably in the visible or long wavelength ultraviolet radiation. The preferred polymerizable regions are acrylates, diacrylates, oligoacrylates, methacrylates, dimethacrylates, oligomethoacrylates, or other biologically acceptable photopolymerizable groups.

Other initiation chemistries may be used besides photoinitiation. These include, for example, water and amine

initiation schemes with isocyanate or isothiocyanate containing macromers used as the polymerizable regions.

MACROMER SIZE

These macromers can vary in molecular weight from 0.2-100 kD, depending on the use. The degree of polymerization, and the size of the starting macromers, directly affect the porosity of the resulting membrane. Thus, the size of the macromers are selected according to the permeability needs of the membrane. For purposes of encapsulating cells and tissue in a manner which prevents the passage of antibodies across the membrane but allows passage of nutrients essential for cellular metabolism, the preferred starting macromer size is in the range of 10 kD to 18.5 kD, with the most preferred being around 18.5 kD. Smaller macromers result in polymer membranes of a higher density with smaller pores.

PHOTOINITIATING DYES

The photoinitiating dyes capture light energy and initiate polymerization of the macromers. Any dye can be used which absorbs light having frequency between 320 nm and 900 nm, can form free radicals, is at least partially water soluble, and is non-toxic to the biological material at the concentration used for polymerization. Examples of suitable dyes are ethyl eosin, eosin Y, fluorescein, 2,2-dimethoxy,2-phenylacetophenone, 2-

methoxy,2-phenylacetophenone, camphorquinone, rose bengal, methylene blue, erythrosin, phloxime, thionine, riboflavin and methylene green. The preferred initiator dye is ethyl eosin due to its spectral properties in aqueous solution.

COCATALYST

The cocatalyst is a nitrogen based compound capable of stimulating the free radical reaction. Primary, secondary, tertiary or quaternary amines are suitable cocatalysts, as are any nitrogen atom containing electron-rich molecules. Cocatalysts include, but are not limited to, triethanolamine, triethylamine, ethanolamine, N-methyl diethanolamine, N,N-dimethyl benzylamine, dibenzyl amine, N-benzyl ethanolamine, N-isopropyl benzylamine, tetramethyl ethylenediamine, potassium persulfate, tetramethyl ethylenediamine, lysine, ornithine, histidine and arginine.

RADIATION WAVELENGTH

The radiation used to initiate the polymerization is either longwave UV or visible light, with a wavelength in the range of 320-900 nm. Preferably, light in the range of 350-700 nm, and even more preferred in the range of 365-514 nm, is used. This light can be provided by any appropriate source able to generate the desired radiation, such as a mercury lamp, longwave UV lamp, He-Ne laser, or an argon ion laser.

THICKNESS AND CONFORMATION OF POLYMER LAYER

Membrane thickness affects a variety of parameters, including perm-selectivity, rigidity, and size of the membrane. In the interfacial polymerization method, the duration of the radiation can be varied to adjust the thickness of the polymer membrane formed. This correlation between membrane thickness and duration of irradiation occurs because the photoinitiator diffuses at a steady rate, with diffusion being a continuously occurring process. Thus, the longer the duration of irradiation, the more photoinitiator will initiate polymerization in the macromer mix, the more macromer will polymerize, and a thicker coat will be formed. Additional factors which affect membrane thickness are the number of reactive groups per macromer, the concentration of accelerators in the macromer solution. This technique allows the creation of very thin membranes because the photoinitiator is first present in a very thin layer at the surface of the biological material, and polymerization only occurs where the photoinitiator is present.

The suspension polymerization method forms a somewhat thicker membrane than the interfacial polymerization method. This is because polymerization occurs in the suspension method throughout the macromer mix. The thickness of membranes formed by the suspension method is determined in part by the viscosity of the macromer solution, the concentration of the macromer in that solution, the fluid mechanical environment of the suspension

and surface active agents in the suspension. These membranes vary in thickness from 50-300 microns. The shape of the structure formed by suspension polymerization can be controlled by shaping the reaction mix prior to polymerization. Spheres can be formed by emulsion with a non-miscible liquid such as oil, coextrusion with such a liquid, or coextrusion with air. Cylinders may be formed by casting or extrusion, and slabs and discoidal shapes can be formed by casting. Additionally, the shape may be formed in relationship to an internal supporting structure such as a screening network of stable polymers (e.g. an alginate gel, preferably high-G alginate, or a woven polymer fiber) or nontoxic metals.

The overall amount, thickness, and rigidity of the membrane formed depends on the interaction of several parameters, including the size and intensity of the radiation beam, duration of exposure of the solution to the radiation, reactivity of the initiator selected, macromer molecular weight, and macromer concentration.

APPLICATIONS FOR THE MACROMERS

The invention can be used for a variety of purposes, some of which are enumerated below, along with benefits which accrue from the use of the invention. Some additional purposes are illustrated by the Examples which follow.

a. Microencapsulating cells: more biocompatible, stronger, more stable, better control of permselectivity, less toxic conditions

b. Macroencapsulating cells: more biocompatible, stronger, more stable, better control of permselectivity, less toxic conditions, easier to incorporate internal or external supporting structure

c. Microencapsulating or macroencapsulating other tissues, with the same benefits

d. Microencapsulating or macroencapsulating pharmaceuticals: more biocompatible, less damaging to the pharmaceutical

e. Coating devices: ease of application, more biocompatible

f. Coating microcapsules: more biocompatible, strengthens them, ease of coating

g. Coating macrocapsules, microcapsules, microspheres and macrospheres: more biocompatible, ease of coating

h. Coating tissues to alter adhesion of other tissues: ease of coating, less toxicity to the tissues, conformal coating versus nonconformal

i. Adhesive between two tissues: ease of adhesion, rapidity of forming adhesive bond, less toxicity to tissues

Applications for the Biodegradable Macromers

Further, the biodegradable macromers can be used for the following purposes.

Prevention of Surgical Adhesions

A preferred application is a method of reducing formation of adhesions after a surgical procedure in a patient. The method includes coating damaged tissue surfaces in a patient with an aqueous solution of a light-sensitive free-radical polymerization initiator and a macromer solution as described above. The coated tissue surfaces are exposed to light sufficient to polymerize the macromer. The light-sensitive free-radical polymerization initiator may be a single compound (e.g., 2,2-dimethoxy-2-phenyl acetophenone) or a combination of a dye and a cocatalyst (e.g., ethyl eosin and triethanol amine).

Controlled drug delivery

A second preferred application concerns a method of locally applying a biologically active substance to tissue surfaces of a patient. The method includes the steps of mixing a biologically active substance with an aqueous solution including a light-sensitive free-radical polymerization initiator and a macromer as described above to form a coating mixture. Tissue surfaces are coated with the coating mixture and exposed to light sufficient to polymerize the macromer. The biologically active substance can be any of a variety of materials, including proteins, carbohydrates, nucleic acids, and inorganic and organic

biologically active molecules. Specific examples include enzymes, antibiotics, antineoplastic agents, local anesthetics, hormones, antiangiogenic agents, antibodies, neurotransmitters, psychoactive drugs, drugs affecting reproductive organs, and oligonucleotides such as antisense oligonucleotides.

In a variation of the method for controlled drug delivery, the macromers are polymerized with the biologically active materials to form microspheres or nanoparticles containing the biologically active material. The macromer, photoinitiator, and agent to be encapsulated are mixed in an aqueous mixture. Particles of the mixture are formed using standard techniques, for example, by mixing in oil to form an emulsion, forming droplets in oil using a nozzle, or forming droplets in air using a nozzle. The suspension or droplets are irradiated with a light suitable for photopolymerization of the macromer.

<u>Tissue Adhesives</u>

Another use of the polymers is in a method for adhering tissue surfaces in a patient. The macromer is mixed with a photoinitiator or photoinitiator/cocatalyst mixture to form an aqueous mixture and the mixture is applied to a tissue surface to which tissue adhesion is desired. The tissue surface is contacted with the tissue with which adhesion is desired, forming a tissue junction. The tissue junction is then irradiated until the macromers are polymerized.

Tissue Coatings

In a particularly preferred application of these macromers, an ultrathin coating is applied to the surface of a tissue, most preferably the lumen of a tissue such as a blood vessel. One use of such a coating is in the treatment or prevention of restenosis, abrupt reclosure, or vasospasm after vascular intervention. The photoinitiator is applied to the surface of the tissue, allowed to react, adsorb or bond to tissue, the unbound photoinitiator is removed by dilution or rinsing, and the macromer solution is applied and polymerized. As demonstrated below, this method is capable of creating uniform polymeric coating of between one and 500 microns in thickness, most preferably about twenty microns, which does not evoke thrombosis or localized inflammation.

Tissue Supports

The macromers can also be used to create tissue supports by forming shaped articles within the body to serve a mechanical function. Such supports include, for example, sealants for bleeding organs, sealants for bone defects and space-fillers for vascular aneurisms. Further, such supports include strictures to hold organs, vessels or tubes in a particular position for a controlled period of time.

The invention described herein is further exemplified in the following Examples. While these Examples provide a

variety of combinations useful in performing the methods of the invention, they are illustrative only and are not to be viewed as limiting in any manner the scope of the invention.

Example 1

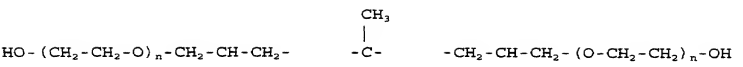
Synthesis of PEG 6 kD Diacrylate

PEG acrylates of molecular weights 400 Da and 1,000 Da ware commercially available from Sartomer and Dajac Inc., respectively. PEG 6 kD (20 g) was dissolved in 200 mL dichloromethane in a 250 mL round bottom flask. The flask was cooled to 0°C and 1.44 mL of triethyl amine and 1.3 mL of acryloyl chloride were added with constant stirring under a dry nitrogen atmosphere. The reaction mixture was then brought to room temperature and stirred for 12 hr under a nitrogen atmosphere. It was then filtered, and the filtrate was precipitated by adding to a large excess of hexane. The crude monomer was purified by dissolving in dichloromethane and precipitating in hexane. Yield 69%.

Example 2

Synthesis of PEG 18.4 kD Tetraacrylate

A tetrafunctional water soluble PEG (30 g; m.w. 18.5 kD) having the following structure was purchased from Polysciences, Inc.:



where F_1 = CONH, COO or NHCOO

X = H, CH_3, C_2H_5, C_6H_5, Cl, Br, OH or CH_2COOH

F_2 = COO, CONH, O or C_6H_4, AND

R = CH_2 or -alkyl-.

The PEG was dried by dissolving in benzene and distilling off the water-benzene azeotrope. PEG 18.5 kD (59 g) was dissolved in 300 mL of benzene in a 500 mL flask. To this, 3.6 mL of triethylamine and 2.2 mL of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was refluxed for 2 hours. It was then cooled and stirred overnight. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in a large excess of hexane. The polymer was further purified by dissolving in methylene chloride and reprecipitating in hexane. The polymer was dried at 50°C under vacuum for 1 day. Yield 68%.

Example 3

Coating of Islet-Containing Alginate-PLL

Microspheres by Surface Dye Adsorption

The microcapsule interfacial polymerization method was used to form membrane around alginate-PLL microcapsules

containing islets. Alginate-PLL coacervated microspheres, containing one or two human pancreatic islets each, were suspended in a 1.1% $CaCl_2$ solution and aspirated free of excess solution to obtain a dense plug of microspheres. A solution of ethyl eosin (0.04% w/v) was prepared in a 1.1 % $CaCl_2$ solution. This solution was filter-sterilized by passage through a 0.45μm filter. The plug of microspheres was suspended in 10 mL of the eosin solution for 2 min to allow uptake of the dye. The microspheres were then washed four times with fresh 1.1% $CaCl_2$ to remove excess dye. A solution of PEG 18.5 tetraacrylate (2 mL; 23% w/v) containing 100μL of a 3.5% w/v solution of triethanolamine in HEPES buffered saline was added to 0.5 mL of these microspheres. The microspheres were exposed to argon ion laser light for 30 seconds with periodic agitation. The suspension of microspheres was uniformly scanned with the light during this period. The microspheres were then washed with calcium solution and the process was repeated in order to further stabilize the coating.

A static glucose stimulation test (SGS) was performed on islets encapsulated in the microspheres coated with PEG gel. Data for insulin secretion in response to this challenge appears in Table 1. The islets were seen to be viable by dithizone staining. The SGS test data confirm the vitality and functionality of the islets.

TABLE 1

SGS

Glucose Concentration (mg%)	initial 60	pulse 300	subsequent 60
	Insulin/Islet/hr (μU/mL)*		
Diffusion Overcoat Method	1.0	10.04±3.56	2.5450.76
Mineral Oil Overcoat Method	1.0	10.23±3.28	1.0250.78
Free Islet Control	1.0	3.74±1.4	1.950.17

* Values are mean ± S.D., all are normalized as compared to the initial 60 mg %, after subjection to the 300 mg % glucose, the islets were resubjected to the initial dose.

PEG diacrylate macromers may be polymerized identically as the PEG tetraacrylate macromer described in this example.

Example 4

Coating Islet-Containing Alginate-PLL Microspheres by the Microcapsule Suspension Polymerization Method

This method takes advantage of the hydrophilic nature of PEG monomers. Alginate/PLL microspheres (2 mL), containing one or two human pancreatic islets each, were mixed with PEG tetraacrylate macromer solution (PEG mol wt 18.5 kD, 23% solution in saline) in a 50 mL transparent centrifuge tube. Triethanolamine (0.1M) and 0.5 mM ethyl eosin were mixed with macromer solution. The excess of macromer solution was decanted,

20 mL of mineral oil was added to the tube, and the reaction mixture was vortexed thoroughly for 5 minutes. Silicone oil will perform equally well in this synthesis but may have poorer adjuvant characteristics if there is any carry-over. Any other water-immiscible liquid may be used as the "oil" phase. Acceptable triethanolamine concentrations range from about 1 mM to about 100mM. Acceptable ethyl eosin concentrations range from about 0.01 mM to more than 10 mM.

The beads were slightly red due to the thin coating of macromer/dye solution, and they were irradiated for 20-50 sec with an argon ion laser (power 50-500 mW). Bleaching of the (red) ethyl eosin color suggested completion of the reaction. The beads were then separated from mineral oil and washed several times with saline solution. The entire procedure was carried out under sterile conditions.

A schematic representation of the microsphere coating process in oil is shown in Figure 3. Alginate/polylysine capsules are soluble in sodium citrate at pH 12. When these coated microspheres came in contact with sodium citrate at pH 12, the inner alginate/polylysine coacervate dissolves and a PEG polymeric membrane could still be seen (crosslinked PEG gels are substantially insoluble in all solvents including water and sodium citrate at pH 12). The uncoated control microspheres dissolved completely and rapidly in the same solution.

A static glucose challenge was performed on the islets as in Example 3. Data again appear in Table 1. The islets were seen to be viable and functional.

Example 5

Encapsulation of Islets of Langerhans

This example makes use of the direct interfacial polymerization. Islets of Langerhans isolated from a human pancreas were encapsulated in PEG tetraacrylate macromer gels. 500 islets suspended in RPMI 1640 medium containing 10% fetal bovine serum were pelleted by centrifuging at 100g for 3 min. The pellet was resuspended in 1 mL of a 23% w/v solution of PEO 18.5 kD diacrylate macromer in HEPES buffered saline. An ethyl eosin solution (5 μL) in vinyl pyrrolidone (at a concentration of 0.5%) was added to this solution along with 100 μL of a 5 M solution of triethanolamine in saline. Mineral oil (20 mL) was then added to the tube which was vigorously agitated to form a dispersion of droplets 200-500 μm in size. This dispersion was then exposed to an argon ion laser with a power of 250 mW, emitting at 514 nm, for 30 sec. The mineral oil was then separated by allowing the microspheres to settle, and the resulting microspheres were washed twice with PBS, once with hexane and finally thrice with media.

Figure 4 shows islets of Langerhans encapsulated in a PEO gel. The viability of the islets was verified by an acridine

orange and propidium iodide staining method and also by dithizone staining. In order to test functional normalcy, an SGS test was performed on these islets. The response of the encapsulated islets was compared to that of free islets maintained in culture for the same time period. All islets were maintained in culture for a week before the SGS was performed. The results are summarized in Table 2. It can be seen that the encapsulated islets secreted significantly ($p<0.05$) higher insulin than the free islets. The PEO gel encapsulation process did not impair function of the islets and in fact helped them maintain their function in culture better than if they had not been encapsulated.

TABLE 2

Islet Insulin secretion

Glucose Concentration (mg%)	60	300	60
	Insulin/Islet/hr (μU/mL)*		
Free islets	1.0	3.74+/-1.40	1.9+/-0.17
Encapsulated Islets	1.0	20.81+/-9.36	2.0+/-0.76

*Values are mean +/- S.D., normalized to initial basal level at 60 mg% glucose.

Example 6

Microencapsulation of Animal Cells

PEG diacrylates of different molecular weight were synthesized by a reaction of acryloyl chloride with PEG as in Example 1. A 20 to 30% solution of macromer was mixed with a cell suspension and the ethyl eosin and triethanolamine initiating system before exposing it to laser light through a coextrusion air flow apparatus, Figure 5. Microspheres were prepared by an air atomization process in which a stream of macromer was atomized by an annular stream of air. The air flow rate used was 1,600 cc/min and macromer flow-rate was 0.5 mL/min. The droplets were allowed to fall to a petri dish containing mineral oil and were exposed to laser light for about 0.15 sec each to cause polymerization and make them insoluble in water. Microspheres so formed were separated from the oil and thoroughly washed with PBS buffer to remove unreacted macromer and residual initiator. The size and shape of microspheres was dependent on extrusion rate (0.05 to 0.1 mL/min) and extruding capillary diameter (18 Ga to 25 Ga). The polymerization times were dependent on initiator concentration (ethyl eosin concentration (5μM to 0.5mM), vinyl pyrrolidone concentration (0.0% to 0.1 %), triethanolamine concentration (5 to 100 mM), laser power (10mW to 1W), and macromer concentration (>10% w/v).

A PEG diacrylate macromer of molecular weight 400 Da was used as a 30% solution in PBS, containing 0.1M

triethanolamine as a cocatalyst and 0.5mM ethyl eosin as a photoinitiator. Spheres prepared using this method are shown in Figure 6. The polymerizations were carried out at physiological pH in the presence of air. This is significant since radical polymerizations may be affected by the presence of oxygen, and the acrylate polymerization is still rapid enough to proceed effectively.

The process also works at lower temperatures. For cellular encapsulation, a 23% solution of PEO diacrylate was used with initiating and polymerization conditions as used in the air atomization technique. Cell viability subsequent to encapsulation was checked by trypan blue exclusion assay. Human foreskin fibroblasts (HFF), Chinese hamster ovary cells (CHO-Kl), and a beta cell insuloma line (RiN5F) were found to be viable (more than 95%) after encapsulation. A wide range (> 10%) of PEG diacrylate concentrations may be used equally effectively, as may PEG tetraacrylate macromers.

Example 7

Coating of Animal Cell-Containing Alginate-PLL Microspheres and Individual Cells by Surface Dye Adsorption

Alginate-PLL coacervated microspheres, containing animal cells, were suspended in a 1.1% $CaCl_2$ solution and were aspirated free of excess solution to obtain a dense plug of microspheres. A solution was filter sterilized by passage

through a 0.45 pm filter. The plug of microspheres was suspended in 10 mL of eosin solution for 2 min to allow dye uptake. A solution of PEG 18.5 tetraacrylate (2 mL; 23% w/v) containing 100 μL of a 3.5 w/v solution of triethanolamine in HEPES buffered saline was added to 0.5 mL of these microspheres. The microspheres were exposed to an argon ion laser for 30 seconds with periodic agitation. The suspension of microspheres was uniformly scanned with the laser during this period. The microspheres were then washed with calcium solution and the process was repeated once more in order to attain a stable coating.

In order to verify survival of cells after the overcoat process, cells in suspension without the alginate/PLL microcapsule were exposed to similar polymerization conditions. 1 mL of lymphoblastic leukemia cells (RAJI) ($5X10^5$ cells) was centrifuged at 300 g for 3 min. A 0.04% filter sterilized ethyl eosin solution in phosphate buffered saline (PBS) (1 mL) was added and the pellet was resuspended. The cells were exposed to the dye for 1 min and washed twice with PBS and then pelleted. Triethanolamine solution (10 μL; 0.1M) was added to the pellet and the tube was vortexed to resuspend the cells. 0.5 mL of PEO 18.5 kD tetraacrylate macromer was then mixed along with this suspension and the resulting mixture was exposed to an argon ion laser (514 nm, 50 mW) for 45 sec. The cells were then washed twice with 10 mL saline and once with media (RPMI 1640 with 10%

FCS and 1% antibiotic, antimycotic). A thin membrane of PEO gel may be observed forming around each individual cell.

No significant difference in viability was seen between the control population (93% viable) and the treated cells (95% viable) by trypan blue exclusion. An assay for cell viability and function was performed by adapting the MTT-Formazan assay for the RAJI cells. This assay indicates > 90% survival. Similar assays were performed with two other model cell lines. Chinese hamster ovary cells (CHO-K1) show no significant difference ($p<0.05$) in metabolic function as evaluated by the MTT-Formazan assay. 3T3 mouse fibroblasts also show no significant reduction ($p<0.05$) in metabolic activity.

Example 8

Coating Animal Cell Containing Alginate-PLL Microspheres by the Oil Suspension Method

Using the method described in Example 4, RAJI cells contained in alginate-PLL microspheres were coated with a PEG polymeric membrane. Viability of these cells was checked by trypan blue exclusion and they were found to be more than 95% viable.

Example 9

Coating of Individual Islets of Langerhans by Surface Dye Adsorption

Using the method described in Example 7, ethyl eosin was adsorbed to the surfaces of islets, exposed to a solution of the PEG macromer with triethanolamine, and exposed to light from an argon-ion laser to form a thin PEG polymeric membrane on the surface of the islets. Islet viability was demonstrated by lack of staining with propidium iodide.

Example 10

Biocompatibility of PEO on Microspheres

In vivo evaluation of the extent of inflammatory response to microspheres prepared in Examples 7 and 8 was carried out by implantation in the peritoneal cavity of mice. Approximately 0.5 mL of microspheres were suspended in 5 mL of sterile HEPES buffered saline. A portion of this suspension (2.5 mL) was injected into the peritoneal cavity of ICR male Swiss white mice. The microspheres were recovered after 4 days by conducting a lavage of the peritoneal cavity with 5 mL of 10U heparin/mL PBS. The extent of cellular growth on the microspheres was visually inspected under a phase contrast microscope. The number of unattached cells present in the recovered lavage fluid was counted using a Coulter counter. Figure 7A shows a photograph of alginate-poly(L-lysine)

microspheres explanted after 4 days, while Figure 7B shows similar spheres which had been coated with PEG gel by the dye diffusion precess before implantation. As expected, bilayer alginate-polylysine capsules not containing an outer alginate layer to provide an extreme test of the ability of the PEG gel layer to enhance the biocompatibility of the bilayer membrane, were completely covered with cells due to the highly cell adhesive nature of the PLL surface, whereas the PEG coated microspheres were virtually free of adherent cells. Almost complete coverage of alginate-poly(L-lysine) was expected because polylysine has amino groups on the surface, and positively charged surface amines can interact with cell surface proteoglycans and support cell growth (Reuveny, et al., (1983) Biotechnol. Bioeng., 25:469-480). The photographs in Figure 7B strongly indicate that the highly charged and cell adhesive surface of PLL is covered by a stable layer of PEG gel. The integrity of the gel did not appear to be compromised.

The non-cell-adhesive tendency of these microspheres was evaluated as a percentage of the total microsphere area which appears covered with cellular overgrowth. These results are summarized in Table 3.

TABLE 3

Microsphere Coverage with Cell Overgrowth

Composition of PEG gel	% Cell coverage
18.5 kD	<1
18.5 kD 90%:0.4 kD 10%	<1
18.5 kD 50%:0.4 kD 50%	<1
35k 90%:0.4 kD 10%	5-7
35k 50%: 0.4 kD 50%	<1
Alginate poly(L-lysine)	60-80

An increase in cell count was a result of activation of resident macrophages which secrete chemical factors such as interleukins and induce nonresident macrophages to migrate to the implant site. The factors also attract fibroblasts responsible for collagen synthesis. The variation of cell counts with chemical composition of the overcoat is shown Figure 8 (A-F). It can be seen from the figure that all PEG coated spheres have substantially reduced cell counts. This is consistent with the PEG overcoat generally causing no irritation of the peritoneal cavity.

However, PEG composition does make a difference in biocompatibility, and increasing molecular weights were associated with a reduction in cell counts. This could be due to the gels made from higher molecular weight oligomers having higher potential for steric repulsion due to the longer chain lengths.

Example 11

Permeability of PEO Gels

Bovine serum albumin, human IgG, or human fibrinogen (20 mg) was dissolved in 2 mL of a 23% w/v solution of oligomeric PEO 18.5 kD tetraacrylate in PBS. This solution was laser polymerized to produce a gel 2cm X 2cm X 0.5 cm in size. The diffusion of bovine serum albumin, human IgG and human fibrinogen (mol wt 66 kD, 150 kD and 350 kD respectively) was monitored through the 2cm X 2cm face of these gels using a total protein assay reagent (Biorad). A typical release profile for a PEO 18.5 kD gel is shown in Figure 9. This gel allowed a slow transport of albumin but did not allow IgG and fibrinogen to diffuse. This indicates that these gels are capable of being used as immunoprotective barriers. This is a vital requirement for a successful animal tissue microencapsulation material.

The release profile was found to be a function of crosslink density and molecular weight of the polyethylene glycol segment of the monomer. Figure 10 shows the release of BSA through gels made from 23% solutions of PEO diacrylates and tetraacrylates of 0.4 kD and 18.5 kD, respectively. It is evident that the 18.5 kD gel is freely permeable to albumin while the 0.4 kD gel restricted the diffusion of albumin. The release of any substance from these gels will depend on the crosslink density of the network and will also depend on the motility of

the PEG segments in the network. This effect is also dependent upon the functionality of the macromer. For example, the permeability of a PEG 18.5 kD tetraacrylate gel is less than that of an otherwise similar PEG 20 kD diacrylate gel.

In the case of short PEO chains between crosslinks, the "pore" produced in the network will have relatively rigid boundaries and will be relatively small and so a macromolecule attempting to diffuse through this gel will be predominantly restricted by a sieving effect. If the chain length between crosslinks is long, the chain can fold and move around with a high motility and, besides the sieving effect, a diffusing macromolecule will also encounter a free volume exclusion effect.

Due to these two contrasting effects a straightforward relation between molecular weight cutoff for diffusion and the molecular weight of the starting oligomer is not completely definable. Yet, a desired release profile for a particular protein or a drug such as a peptide may be accomplished by adjusting the crosslink density and length of PEG segments. Correspondingly, a desired protein permeability profile may be arranged to permit the diffusion of nutrients, oxygen, carbon dioxide, waste products, hormones, growth factors, transport proteins, and released cellularly synthesized proteins, while restricting the diffusion of antibodies and complement proteins and also the ingress of cells, to provide immunoprotectivity to transplanted cells or tissue. The three dimensional crosslinked

covalently bonded polymeric network is chemically stable for long-term in vivo applications.

Example 12

Treatment of Silicone Rubber to Enhance Biocompatibility

Pieces of medical grade silicone rubber (2 X 2 cm) were soaked for 1 h in benzene containing 23% 0.4 kD PEG diacrylate and 0.5% 2,2-dimethoxy-2-phenyl acetophenone. The thus swollen rubber was irradiated for 15 min with a long wave UV lamp (365 nm). After irradiation, the sample was rinsed in benzene and dried. The air contact angles of silicone rubber under water were measured before and after treatment. The decreased contact angle of 500 after treatment, over the initial contact angle of 630 for untreated silicone rubber reflects an increased hydrophilicity due to the presence of the PEG gel on the rubber surface.

This technique demonstrates that macromer polymerization can be used to modify a polymer surface so as to enhance biocompatability. For instance, a polyurethane catheter can be treated by this method to obtain an implantable device coated with PEG. The PEG was firmly anchored to the surface of the polyurethane catheter because the macromer was allowed to penetrate the catheter surface (to a depth of 1-2 microns) during the soaking period before photopolymerization. Upon irradiation,

an interpenetrating network of PEG and polyurethane results. The PEG was thereby inextricably intertwined with the polyurethane.

Example 13

Treatment of Polyurethane

INTRACATH (Becton Dickinson) polyurethane intravenous catheters (19 ga) were modified at their outer surfaces with polyethylene glycol diacrylate (PEG DA) of molecular weight 400 and 10000. The prepolymer was dissolved in tetrahydrofuran (THF), a solvent for the polyurethane, at 50°C, where polyurethane dissolution is relatively slow. The following solution was prepared and warmed to 50°C:

PEG DA (MW 400)	15%
PEG DA (MW 10000)	15%
THF	70%

with 2,2-dimethoxy, 2-phenyl actophenone at 1.6% of the above solution.

2.5" length catheter segments were closed at one end by melting a 2 mm length by pressing with a hot metal spatula to from a flat tab. This tab was used to fix the catheter in the vessel wall in subsequent animal experiments. The catheter was held with forceps at the tab end and dipped in the treatment solution for 1-3 sec, pulled out, and the excess fluid shaken off. The treated catheter was illuminated with an ultraviolet light (Black Ray, 360 nm) for 2-3 min, rotating the catheter. An

untreated control was similarly treated in 70% THF with 30% water replacing the PEG in the treatment solution.

Following this treatment, both the treated and control catheters were transferred to 100% methylene chloride to extract unreacted materials; this extraction was carried out for 36 hr with solvent replacement every 6 hr. These catheters were then dried and transferred to 70% ethanol, and then into water before use.

A second composition was also investigated:

PEG DA (MW 400)	10%
PEG DA (MW 10000)	15%
Polyethylene oxide (MW 100,000)	5%
THF	70%

with 2,2-dimethoxy, 2-phenyl acetophenone at 1.6% of the above solution.

In this case, the polyethylene oxide of mw 100,000 was not a prepolymer and was immobilized within the PEG DA matrix by entanglement, rather than by chemical attachment.

Adult New Zealand male rabbits (7-10 lb) were anesthetized with rompun-acepromazien-ketamine. The animal was shaved on the ventrolateral jugular and the vessel was raised. A catheter was inserted into the vessel with the tab outside, and tied in place via the tab with 4.0 nylon to the adventitia. The catheter was inserted 1.5 to 2.0" into the vessel. The skin incision was closed.

After a period of 3 days, the animals were euthanized by overdose of pentobarbital intraperitoneally. The vessel was again raised and flushed with phosphate buffered saline (PBS) to superficially rinse away blood between the catheter and the vessel wall. Two 500 ml bottles, one filled with PBS and one with formalin in PBS were hung from an i.v. pole scaffold, and the hydraulic differential was used to perfusion fix the vessel. The vessels were removed proximal and distal to the ends of the catheters.

The treated catheters were completely wettable, and were very slippery.

A total of 12 rabbits were catheterized for 72 hr. Six were control, unmodified catheters. These catheters could not be removed from the vessel wall without dissection, i.e. they were tightly incorporated into the vessel. These catheters upon removal were red, and the vessel was barely patent. By contrast, the treated catheters were easily removable, the vessels were clearly patent, and the catheters were not red. Under the light microscope, a small amount of white thrombus could be seen on both formulations of the catheter coating, with somewhat lesser amounts on the formulation containing the polyethylene oxide 100,000.

Example 14

Treatment of Ultrafiltration Membranes

The processes of Examples described above can be applied to the treatment of macrocapsular surfaces, such as those used for ultrafiltration, hemodialysis and non-microencapsulated immunoisolation of animal tissue. The macrocapsule in this case will usually be microporous with a molecular weight cutoff below 70,000 Da. It may be in the form of a hollow fiber, a spiral module, a flat sheet or other configuration. The surface of such a macrocapsule can easily be modified using the PEO gel coating process to produce a non-fouling, non-thrombogenic, and non-cell-adhesive surface. The coating serves to enhance biocompatibility and to offer additional immunoprotection. Materials which can be modified in this manner include polysulfones, cellulosic membranes, polycarbonates, polyamides, polyimides, polybenzimidazoles, nylons, and poly(acrylonitrile-co-vinyl chloride) copolymers and the like.

Depending on the physical and chemical nature of the surface a variety of methods can be employed to form biocompatible overcoats. Hydrophilic surfaces can simply be coated by applying a thin layer of a 30% w/v polymerizable solution of PEG diacrylate containing appropriate amounts of dye and amine. Hydrophobic surfaces can be first rendered hydrophilic by gas plasma discharge treatment and the resulting surface can then be similarly coated, or they may simply be

treated with a surfactant before or during treatment with the PEG diacrylate solution.

Example 15

Treatment of Textured Materials and Hydrogels

The surface of materials having a certain degree of surface texture, such as woven dacron, dacron velour, and expanded poly(tetrafluoroethylene) (ePTFE) membranes, was treated using the coating method described herein. Textured and macroporous surfaces allow greater adhesion of the PEG gel to the material surface. This allows the coating of relatively hydrophobic materials such as PTFE and poly(ethylene terepthalate) (PET).

Implantable materials such as enzymatic and ion sensitive electrodes, having a hydrogel (such as poly (HEMA), crosslinked poly(vinyl alcohol) and poly(vinyl pyrrolidone)) on their surface, are coated with the more biocompatible PEO gel in a manner similar to the dye adsorption and polymerization technique used for the alginate-PLL microspheres.

Example 16

Treatment of Dense Materials

The surfaces of dense (e.g., nontextured, nongel) materials such as polymers (including PET, PTFE, polycarbonates, polyamides, polysulfones, polyurethanes, polyethylene,

polypropylene, polystyrene), glass, and ceramics can be treated with PEO gel coatings. Hydrophobic surfaces were initially treated by a gas plasma discharge to render the surface hydrophilic. This ensures better adhesion of the PEO gel coating to the surface. Alternatively, coupling agents may be used to increase adhesion, as readily apparent to those skilled in the art of polymer synthesis.

Example 17

Rate of Polymerization

To demonstrate rapidity of gelation in laser-initiated polymerizations of multifunctional acrylic monomers, the kinetics of a typical reaction were investigated. Trimethylolpropyl triacrylate containing 5 x 10^{-4} M ethyl eosin as a photoinitiator in 10 μmoles of N-vinyl pyrrolidone per mL of macromer mix and 0.1M of triethanolamine as a cocatalyst, was irradiated with a 500 mW argon ion laser (514 nm wavelength, power 3.05x10^5 W/m^2, beam diameter 1 mm, average gel diameter produced 1 mm). A plot of the length of the spike of gel formed by penetration of the laser beam into the gel versus laser irradiation time is shown in Figure 11A. The spikes formed as a result of laser light penetration into the macromer can be seen in Figure 11B.

A 23% w/w solution of various macromers in HEPES buffered saline containing 3 μL of initiator solution (300 mg/mL of 2,2-dimethoxy-2-phenylacetophenone in N-vinyl pyrrolidone) was

used. 100 μL of the solution was placed on a glass coverslip and irradiated with a low intensity long wave UV (LWUV) lamp (BlakRay, model 3-100A with flood). The times required for gelation to occur were noted and are given in Table 4. These times were typically in the range of 10 seconds.

TABLE 4

Gelling Time

Polymer Code	Gel Time (sec) (mean ± S.D.)
0.4 kD	6.9 ± 0.5
1 kD	21.3 ± 2.4
6 kD	14.2 ± 0.5
10 kD	8.3 ± 0.2
18.5 kD	6.9 ± 0.1
20 kD	9.0 ± 0.4

Time periods of about 10-100 ms were sufficient to gel a 300 μm diameter droplet (a typical size of gel used in microencapsulation technology). This rapid gelation, if used in conjunction with proper choice of macromers, can lead to entrapment of living cells in a three dimensional covalently bonded polymeric network. The monochromatic laser light will not be absorbed by the cells unless a proper chromophore is present, and is considered to be harmless if wavelength is more than about 400 nm. Exposure to long wavelength ultraviolet light (>360 nm) is harmless at practical intensities and durations.

Example 18

PEO Gel Interactions

Biocompatibility with HFF (human foreskin fibroblasts) cells was demonstrated as follows.

HFF cells were seeded on PEO 18.5 kD tetraacrylate gels at a density of 18,000 cells/cm² in Dulbecco's modification of Eagle's medium containing 10% fetal calf serum. The gels were then incubated at 37°C in a 5% CO_2 environment for 4 hr. At the end of this time the gels were washed with PBS to remove any non-adherent cells and were observed under a phase contrast microscope at a magnification of 200X. Figure 12A shows the growth of these cells on a typical PEG gel as compared to glass surface (Figure 12B). The number of attached cells/cm² was found to be 510 ± 170 on the gel surfaces as compared to 13,200 ± 3,910 for a control glass surface. The cells on these gels appeared rounded and were not in their normal spread morphology, strongly indicating that these gels do not encourage cell attachment.

Biocompatibility on microspheres was demonstrated as follows. Figure 13 shows a photograph of microspheres explanted from mice as in Example 10; after 4 days very little fibrous overgrowth was seen. The resistance of PEG chains to protein adsorption and hence cellular growth was well documented. Table 5 summarizes the extent of cellular overgrowth seen on these microspheres after 4 day intraperitoneal implants for various PEG diacrylate gels.

TABLE 5

PEG Diacrylate for Gels (mol wt, Daltons)	Extent of Cellular Overgrowth
400	5-10%
1,000	15-25%
5,000	3-5%
6,000	2-15%
10,000	10-20%
18,500	4-10%

Example 19

Characterization and Mechanical Analysis of PEO Gels

Solutions of PEO diacrylates (23% w/v; 0.4 kD, 6 kD, 10 kD) and PEG tetraacrylates (18.5 kD) were used. An initiator solution (10 μL) containing 30 mg/mL of 2,2-dimethoxy-2-phenyl acetophenone in vinyl-2-pyrrolidone was used per mL of the macromer solution. The solution of initiator containing macromer was placed in a 4.0 X 1.0 X 0.5 cm mold and exposed to a long wave ultraviolet lamp (365 nm) for approximately 10 seconds to induce gelation. Samples were allowed to equilibrate in phosphate buffered saline (pH 7.4) for 1 week before analysis 1 performed.

A series of "dogbone" samples (samples cut from a slab into the shape of a dogbone, with wide regions at both ends and a narrower long region in the middle) were cut for ultimate tensile strength tests. Thickness of the samples was defined by the thickness of the sample from which they were cut. These thicknesses ranged from approximately 0.5 mm to 1.75 mm. The samples were 20 mm long and 2 mm wide at a narrow "neck" region. The stress strain tests were run in length control at a rate of 4% per second. After each test, the cross sectional area was determined. Table 6 shows the ultimate tensile strength data. It is seen that the lower molecular weight macromers in general give stronger gels which were less extensible than those made using the higher molecular weight macromers. The PEG 18.5 kD tetraacrylate gel is seen to be anomalous in this series, resulting from the multifunctionality of the macromer and the corresponding higher crosslinking density in the resulting gel. This type of strengthening result could be similarly achieved with macromers obtained having other than four free radical sensitive groups, such as acrylate groups.

TABLE 6

Gel strength Tests

	PEO Acrylate Precursor Molecular Weight			
	0.4 kD	6 kD	10 kD	18.5 kD
Stress (kPa)*	168+/-51	98+/-15	33+/-7	115+/-56
% Strain*	8+/-3	71+/-13	110+/-9	40+/-15
Slope*	22+/-5	1.32+/-0.31	0.27+/-0.04	2.67+/-0.55

*Values are mean+/-S.D.

For the creep tests, eight samples approximately 0.2 X 0.4 X 2 cm were loaded while submersed in saline solution. They were tested with a constant unique predetermined load for one hour and a small recovery load for ten minutes. Gels made from PEG diacrylates of 1 kD, 6 kD, and 10 kD, and PEG tetraacrylates of 18.5 kD PEO molecular weight were used for this study. The 10 kD test was terminated due to a limit error (the sample stretched beyond the travel of the loading frame). The 1 kD sample was tested with a load of 10g and a recovery load of 0.2g. The 6 kD sample was tested at a load of 13g with a recovery load of 0.5g. The 18.5 kD sample was tested at a load of 13g with a recovery load of 0.2g. The choice of loads for these samples produced classical creep curves with primary and secondary regions. The

traces for creep for the 1 kD, 6 kD, and 18.5 kD samples appear in Figure 14A-C, respectively.

Example 20

Water Content of PEO Gels

Solutions of various macromers were made as described above. Gels in the shape of discs were made using a mold. The solutions (400 μL) was used for each disc. The solutions were irradiated for 2 minutes to ensure thorough gelation. The disc shaped gels were removed and dried under vacuum at 60°C for 2 days. The discs were weighed (WI) and then extracted repeatedly with chloroform for 1 day. The discs were dried again and weighed (W2). The gel fraction was calculated as W2/W1. This data appears in Table 7.

Determination of Degree of Hydration

Subsequent to extraction, the discs were allowed to equilibrate with HBS for 6 hours and weighed (W3) after excess water had been carefully swabbed away. The total water content was calculated as (W3-W2) X 100/W3. The data for gel water contents is summarized in the following table.

TABLE 7

Polymer Coat	% Total Water	% Gel Content
0.4 kD	-	99.8 ± 1.9
1 kD	79.8 ± 2.1	94.5 ± 2.0
6 kD	95.2 ± 2.5	69.4 ± 0.6
10 kD	91.4 ± 1.6	96.9 ± 1.5
18.5 kD	91.4 ± 0.9	80.3 ± 0.9
20 kD	94.4 ± 0.6	85.0 ± 0.4

Example 21

Mechanical Stability of PEO Gels after Implantation

PEG diacrylate (10 kD) and PEG tetraacrylate (18.5 kD) were cast in dogbone shapes as described in Example 19. PEG--dacrylate or tetraacrylate (23% w/w) in sterile HEPES buffered saline (HBS) (0.9% NaCl, 10 - HEPES, pH 7.4) containing 900 ppm of 2,2-dimethoxy-2-phenoxyacetophenone as initiator, was poured into an aluminum mold and irradiated with a LWUV lamp (Black ray) for 1 min. The initial weights of these samples were found after oven-drying these gels to constant weight. The samples were soxhlet-extracted with methylene chloride for 36 hours in order to leach out any unreacted prepolymer from the gel matrix (sol-leaching) prior to testing. The process of extraction was continued until the dried gels gave constant weight.

ICR Swiss male white mice, 6-8 weeks old (Sprague-Dawley), were anesthetized by an intraperitoneal injection of

sodium pentobarbital. The abdominal region of the mouse was shaved and prepared with betadine. A ventral midline incision 10-15 mm long was made. The polymer sample, fully hydrated in sterile PBS (Phosphate buffered saline) or HEPES buffered saline (for calcification studies), was inserted through the incision and placed over the mesentery, away from the wound site. The peritoneal wall was closed with a lock stitched running suture (4.0 silk, Ethicon). The skin was closed with stainless steel skin staples, and a topical antibiotic (Furacin) was applied over the incision site. Three animals were used for each time point. One dogbone sample was implanted per mouse and explanted at the end of 1 week, 3 weeks, 6 weeks, and 8 weeks. Explanted gels were rinsed in HBS twice and then treated with 0.3 mg/mL pronase (Calbiochem) to remove any adherent cells and tissue. The samples were then oven-dried to a constant weight, extracted, and reswelled as mentioned before.

Tensile stress strain test was conducted on both control (unimplanted) and explanted dogbones in a small horizontal Instron-like device. The device is an aluminum platform consisting of two clamps mounted flat on a wooden board between two parallel aluminum guide. The top clamp was stationary while the bottom clamp was movable. Both the frictional surfaces of the moving clamp and the platform were coated with aluminum backed Teflon (Cole-Parmer) to minimize frictional resistance. The moving clamp was fastened to a device

capable of applying a gradually increasing load. The whole set up was placed horizontally under a dissecting microscope (Reichert) and the sample elongation was monitored using a video camera. The image from the camera was acquired by an image processor (Argus-10, Hamamatsu) and sent to a monitor. After breakage, a cross section of the break surface was cut and the area measured. The load at break was divided by this cross section to find the maximum tensile stress. Table 8 lists the stress at fracture of PEG tetraacrylate (18.5 kD) hydrogels explanted at various time intervals. No significant change in tensile strength was evident with time. Thus, the gels appear mechanically stable to biodegradation in vivo within the maximum time frame of implant in mice.

TABLE 8

TIME IMPLANTED	STRESS (KPa) (mean ± error*)	STRAIN AV. (mean ± error*)
1 WK	52.8 ± 16.7	0.32 ± 0.19
3 WK	36.7 ± 10.6	0.37 ± 0.17
6 WK	73.3 ± 34.9	0.42 ± 0.26
8 WK	34.1†	0.30†
CONTROL	44.9 ± 5.3	0.22 ± 0.22

* Error based on 90% confidence limits.

† Single sample.

Example 22

Monitoring of Calcification of PEO Gels

Disc shaped PEG-tetraacrylate hydrogels (m.w. 18.5 kD) were implanted intraperitoneally in mice as mentioned above for a period of 1 week, 3 weeks, 6 weeks, or 8 weeks. Explanted gels were rinsed in HBS twice and treated with Pronase (Calbiochem) to remove cells and cell debris. The samples were then equilibrated in HBS to let free Ca^{++} diffuse out from the gel matrix. The gels were then oven-dried (Blue-M) to a constant weight and transferred to Aluminum oxide crucibles (COORS, high temperature resistant). They were incinerated in a furnace at 700°C for at least 16 hours. Crucibles were checked for total incineration, if any residual remnants or debris was seen they were additionally incinerated for 12 hours. Subsequently, the crucibles were filled with 2 mL of 0.5 M HCl to dissolve Ca^{++} salt and other minerals in the sample. This solution was filtered and analyzed with atomic absorption spectroscopy (AA) for calcium content.

Calcification data on PEG-tetraacrylate (mol. wt. 18.5 kD) gel implants is given in Table 9. No significant increase in calcification was observed up to an 8 week period of implantation in mice.

TABLE 9

TIME (Days)	CALCIFICATION (mean ± error*) (mg Calcium/g of Dry gel wt.)
7	2.33 ± 0.20
21	0.88 ± 0.009
42	1.08 ± 0.30
56	1.17 ± 0.26

* Error based on 90% confidence limits.

Example 23

Encapsulation of Neurotransmitter-Releasing Cells

Paralysis agitans, more commonly called Parkinson's disease, is characterized by a lack of the neurotransmitter dopamine within the striatum of the brain. Dopamine secreting cells such as cells from the ventral mesencephalon, from neuroblastoid cell lines or from the adrenal medulla can be encapsulated in a manner similar to that of other cells mentioned in prior Examples. Cells (including genetically engineered cells) secreting a precursor for a neurotransmitter, an agonist, a derivative or a mimic of a particular neurotransmitter or analogs can also be encapsulated.

Example 24

Encapsulation of Hemoglobin for Synthetic Erythrocytes

Hemoglobin in its free form can be encapsulated in PEG gels and retained by selection of a PEG chain length and cross-link density which prevents diffusion. The diffusion of hemoglobin from the gels may be further impeded by the use of polyhemoglobin, which is a cross-linked form of hemoglobin. The polyhemoglobin molecule is too large to diffuse from the PEG gel. Suitable encapsulation of either native or crosslinked hemoglobin may be used to manufacture synthetic erythrocytes. The entrapment of hemoglobin in small spheres (< 5μm) of these highly biocompatible materials would lead to enhanced circulation times relative to crosslinked hemoglobin or liposome encapsulated hemoglobin.

Hemoglobin in PBS is mixed with the prepolymer in the following formulation:

Hemoglobin at the desired amount	
PEG DA (MW 10000)	35%
PEG DA (MW 1000)	5%
PBS	60%

with 2,2-dimethoxy, 2-phenyl acetophenone at 1.6% of the above solution.

This solution is placed in mineral oil at a ratio of 1 part hemoglobin/prepolymer solution to 5 parts mineral oil and is rapidly agitated with a motorized mixer to form an emulsion.

This emulsion is illuminated with a long-wavelength ultraviolet light (360nm) for 5 min to crosslink the PEG prepolymer to form a gel. The mw of the prepolymer may be selected to resist the diffusion of the hemoglobin from the gel, with smaller PEG DA molecular weights giving less diffusion. PEG DA of MW 10000, further crosslinked with PEG DA 1000, should possess the appropriate permselectivity to restrict hemoglobin diffusion, and it should possess the appropriate biocompatibility to circulate within the bloodstream.

Example 25

Entrapment of Enzymes for Correction of Metabolic Disorders and Chemotherapy

Congenital deficiency of the enzyme catalase causes acatalasemia. Immobilization of catalase in PEG gel networks could provide a method of enzyme replacement to treat this disease. Entrapment of glucosidase can similarly be useful in treating Gaucher's disease. Microspherical PEG gels entrapping urease can be used in extracorporeal blood to convert urea into ammonia. Enzymes such as asparaginase can degrade amino acids needed by tumor cells. Immunogenicity of these enzymes prevents direct use for chemotherapy. Entrapment of such enzymes in immunoprotective PEG gels, however, can support successful chemotherapy. A suitable formulation can be developed for either slow release or no release of the enzyme.

Catalase in PBS is mixed with the prepolymer in the following formulation:

Catalase at the desired amount	
PEG DA (MW 10000)	35%
PEG DA (MW 1000)	5%
PBS	60%

with 2,2-dimethoxy, 2-phenyl acetophenone at 1.6% of the above solution.

This solution is placed in mineral oil at a ratio of 1 part catalase/prepolymer solution to 5 parts mineral oil and is rapidly agitated with a motorized mixer to form an emulsion. This emulsion is illuminated with a long-wavelength ultraviolet light (360nm) for 5 min to crosslink the PEG prepolymer to form a gel. The mw of the prepolymer may be selected to resist the diffusion of the catalase from the gel, with smaller PEG DA molecular weights giving less diffusion.

PEG DA of MW 10,000, further crosslinked with PEG DA 1000, should possess the appropriate permselectivity to restrict catalase diffusion, and it should possess the appropriate permselectivity to permit the diffusion of hydrogen peroxide into the gel-entrapped catalase to allow the enzymatic removal of the hydrogen peroxide from the bloodstream. Furthermore, it should possess the appropriate biocompatibility to circulate within the bloodstream.

In this way, the gel is used for the controlled containment of a bioactive agent within the body. The active agent (enzyme) is large and is retained within the gel, and the agent upon which it acts (substrate) is small and can diffuse into the enzyme rich compartment. However, the active agent is prohibited from leaving the body or targeted body compartment because it cannot diffuse out of the gel compartment.

Example 26

Use of PEO Gels as Adhesive to Rejoin Severed Nerve

A formulation of PEG tetraacrylate (10%, 18.5K), was used as adhesive for stabilizing the sutureless apposition of the ends of transected sciatic nerves in the rat. Rats were under pentobarbital anesthesia during sterile surgical procedures. The sciatic nerve was exposed through a lateral approach by deflecting the heads of the biceps femoralis at the mid-thigh level. The sciatic nerve was mobilized for approximately 1 cm and transected with iridectomy scissors approximately 3 mm proximal to the tibial-peroneal bifurcation. The gap between the ends of the severed nerves was 2-3 mm. The wound was irrigated with saline and lightly swabbed to remove excess saline. Sterile, unpolymerized PEG tetraacrylate solution was applied to the wound. Using delicate forceps to hold the adventitia or perineurium, the nerve ends were brought into apposition, the

macromer solution containing 2,2-dimethoxy-2-phenoxyacetophenone as a photoinitiator applied to the nerve ends and the wound was exposed to long wavelength UV-light (365 nm) for about 10 sec to polymerize the adhesive. The forceps were gently pulled away. Care was taken to prevent the macromer solution from flowing between the two nerve stumps. Alternatively, the nerve stump junction was shielded from illumination, *e.g.*, with a metal foil, to prevent gelation of the macromer solution between the stumps; the remaining macromer solution was then simply washed away.

In an alternative approach, both ends of the transected nerve can be held together with one pair of forceps. Forceps tips are coated lightly with petrolatum to prevent reaction with the adhesive.

The polymerized adhesive serves to encapsulate the wound and adhere the nerve to the underlying muscle. The anastomosis of the nerve ends resists gentle mobilization of the joint, demonstrating a moderate degree of stabilization. The muscle and skin were closed with sutures. Re-examination after one month shows that severed nerves remain reconnected, despite unrestrained activity of the animals.

Example 27

Surgical Adhesive

Abdominal muscle flaps from female New Zealand white rabbits were excised and cut into strips 1 cm X 5 cm. The flaps were approximately 0.5 to 0.8 cm thick. The lap joint, 1 cm X 1 cm, was made using two such flaps. Two different PEO di- and tetra-acrylate macromer compositions, 0.4K (di-) and 18.5K (tetra-), were evaluated. The 0.4K composition was a viscous liquid and was used without further dilution. The 18.5K composition was used as a 23% w/w solution in HBS. 125 µl of ethyl eosin solution in n-vinyl pyrrolidone (20 mg/ml) along with 50 µl of triethanolamine was added to each ml of the adhesive solution. 100 µl of adhesive solution was applied to each of the overlapping flaps. The lap joint was then irradiated by scanning with a 2 W argon ion laser for 30 seconds from each side. The strength of the resulting joints was evaluated by measuring the force required to shear the lap joint. One end of the lap joint was clamped and an increasing load was applied to the other end, while holding the joint horizontally until it failed. Four joints were tested for each composition. The 0.4K joints had a strength of 12.0 ± 6.9 KPa (mean ± S.D.), while the 18.5K joints had a strength of 2.7 ± 0.5 KPa. It is significant to note that it was possible to achieve photopolymerization and reasonable joint strength despite the 6-8 mm thickness of tissue. A

spectrophotometric estimate using 514 nm light showed less than 1% transmission through such muscle tissue.

Example 28

Modification of polyvinyl alcohol

2 g of polyvinyl alcohol (mol wt 100,000-110,000) was dissolved in 20 ml of hot DMSO. The solution was cooled to room temperature and 0.2 ml of triethylamine and 0.2 ml of acryloyl chloride was added with vigorous stirring, under an argon atmosphere. The reaction mixture was heated to 70°C for 2 hr and cooled. The polymer was precipitated in acetone, redissolved in hot water and precipitated again in acetone. Finally it was dried under vacuum for 12 hr at 60°C. 5-10% w/v solution of this polymer in PBS was mixed with the UV photoinitiator and polymerized using long wavelength UV light to make microspheres 200-1,000 microns in size.

These microspheres were stable to autoclaving in water, which indicates that the gel is covalently cross-linked. The gel is extremely elastic. This macromer, PVA multiacrylate, may be used to increase the crosslinking density in PEG diacrylate gels, with corresponding changes in mechanical and permeability properties. This approach could be pursued with any number of water-soluble polymers chemically modified with photopolymerizable groups, for example with water-soluble polymers chosen from polyvinylpyrrolidone, polyethyloxazoline,

polyethyleneoxide-polypropyleneoxide copolymers, polysaccharides such as dextran, alginate, hyaluronic acid, chondroitin sulfate, heparin, heparin sulfate, heparan sulfate, guar gum, gellan gum, xanthan gum, carrageenan gum, and proteins, such as albumin, collagen, and gelatin.

Example 29

Use of Alternative Photopolymerizable Moieties

Many photopolymerizable groups may be used to enable gelation. To illustrate a typical alternative synthesis, a synthesis for PEG 1K urethane methacrylate is described as follows:

In a 250 ml round bottom flask, 10 g of PEG 1K diol was dissolved in 150 ml benzene. 3.38 g of 2-isocyanatoethylmethacrylate and 20 μl of dibutyltindilaurate were slowly introduced into the flask. The reaction was refluxed for 6 hours, cooled and poured into 1000 ml hexane. The precipitate was then filtered and dried under vacuum at 60°C for 24 hours. In this case, a methacrylate free radical polymerizable group was attached to the polymer via a urethane linkage, rather than an ester link as is obtained, e.g. when reacting with aryloxyl chloride.

Example 30

Formation of Alginate-PLL-alginate Microcapsules with Photopolymerizable Polycations

Alginate-polylysine-alginate microcapsules are made by adsorbing, or coacervating, a polycation, such as polylysine (PLL), upon a gelled microsphere of alginate. The resulting membrane is held together by charge-charge interactions and thus has limited stability. To increase this stability, the polycation can be made photopolymerizable by the addition of a carbon-carbon double bond, for example. This can be used to increase the stability of the membrane by itself, or to react, for example, with photopolymerizable PEG to enhance biocompatibility.

To illustrate the synthesis of such a photopolymerizable polycation, 1 g of polyallylamine hydrochloride was weighed in 100 ml glass beaker and dissolved in 10 ml distilled water (DW). The pH of the polymer solution was adjusted to 7 using 0.2 M sodium hydroxide solution. The polymer was then separated by precipitating in a large excess of acetone. It was then redissolved in 10 ml DW and the solution was transferred to 50 ml round bottom flask. 0.2 ml glycidyl methacrylate was slowly introduced into the reaction flask and the reaction mixture was stirred for 48 hours at room temperature. The solution was poured into 200 ml acetone and the precipitate was separated by filtration and dried in vacuum.

This macromer is useful in photochemically stabilizing an alginate-PLL-alginate, both in the presence or in the absence of a second polymerizable species such as a PEG diacrylate.

In addition to use in encapsulating cells in materials such as alginate, such photopolymerizable polycations may be useful as a primer or coupling agent to increase polymer adhesion to cells, cell aggregates, tissues and synthetic materials, by virtue of adsorption of the photopolymerizable polymer bonding to the PEG photopolymerizable gel.

Example 31

Cellular Microencapsulation for Evaluation of Anti-Human Immunodeficiency Virus Drugs In Vivo

HIV infected or uninfected human T-lymphoblastoid cells can be encapsulated into PEG gels as described for other cells above. These microcapsules can be implanted in a nonhuman animal and then treated with test drugs such as AZT or DDI. After treatment, the microcapsules can be harvested and the encapsulated cells screened for viability and functional normalcy using a fluorescein diacetate/ethidium bromide live/dead assay. Survival of infected cells indicates successful action of the drug. Lack of biocompatibility is a documented problem in this approach to drug evaluations, but the highly biocompatible gels described herein solve this problem.

Example 32

Use of Alternative

Photoinitiator/Photosensitizer Systems

It is possible to initiate photopolymerization with a wide variety of dyes as initiators and a number of electron donors as effective cocatalysts. Table 10 illustrates photopolymerization initiated by several other dyes which have chromophores absorbing at widely different wavelengths. All gelations were carried out using a 23% w/w solution of 18.5 kD PEG tetraacrylate in HEPES buffered saline. These initiating systems compare favorably with conventional thermal initiating systems, as can also be seen from Table 10.

Table 10

Polymerization Initiation

INITIATOR	LIGHT SOURCE*	TEMPERATURE °C	APPROXIMATE GEL TIME, (SEC)
Eosin Y, 0.00015M, Triethanolamine 0.65M	S1 with UV filter	25	10
Eosin Y, 000015M; Triethanolamine 0.65M	S4	25	0.1
Methylene Blue, 0.00024M; p-toluenesulfonic acid, 0.0048M	S3	25	120
2,2-dimethoxy-2-phenyl acetophenone 900 ppm	S2	25	8
Potassium Persulfate 0.0168M	-	75	180
Potassium Persulfate 0.0168M; tetramethyl ethylene-diamine 0. 039M	-	25	120
Tetramethyl ethylene-diamine 0.039M; Riboflavin 0.00047M	S1 with UV filter	25	300

* LIST OF LIGHT SOURCES USED

CODE	SOURCE
S1	Mercury lamp, LEITZ WETZLER Type 307-148.002, 100W
S2	Black Ray longwave UV lamp, model B-100A W/FLOOD
S3	MELLES GRIOT He-Ne laser, 10mW output, 1=632 nm
S4	American laser corporation, argon ion laser, model 909BP-15-01001; 1=488 and 514 nm

Numerous other dyes can be used for photopolymerization. These dyes include but are not limited to Erythrosin, phloxime, rose bengal, thionine, camphorquinone,

ethyl eosin, eosin, methylene blue, and riboflavin. Possible cocatalysts that can be used include but are not limited to: N-methyl diethanolamine, N,N-dimethyl benzylamine, triethanolamine, triethylamine, dibenzyl amine, N-benzyl ethanolamine, N-isopropyl benzylamine.

BIODEGRADABLE MACROMERS

Table 11 shows the code names of the various macromers synthesized in or for use in the examples, along with their composition in terms of the molecular weight of the central PEG segment and the degree of polymerization of the degradable comonomer.

Table 11: Macromer Molecular Weight and Composition

PEG molecular	Comonomer	D.P. of conomoner per OH group	Polymer weight Code
20,000	glycolide	15	20KG
18,500	glycolide	2.5	18.5K
10,000	glycolide	7	10KG
6,000	glycolide	5	6KG
4,000	glycolide	5	4KG
1,000	glycolide	2	1KG
20,000	DL-lactide	10	20KL
18,500	DL-lactide	10	18.5KL
10,000	DL-lactide	5	10KL
6,000	DL-lactide	5	6KL
1,000	DL-lactide	2	1KL
600	DL-lactide	2	0.6KL
600	DL-lactide + caprolactone(CL)	lactide 2; CL 1	0.6KLCL
18,500	caprolactone	2.5	18.5KCL
18,500	-	-	18.5KCO

Example 33

Synthesis of Photopolymerized Biodegradable Hydrogels.

PEG-based hydrogels

PEG-based biodegradable hydrogels are formed by the rapid laser or UV photopolymerization of water soluble macromers. Macromers, in turn, are synthesized by adding glycolic acid oligomers to the end groups of PEG and then capping with acrylic end groups. The PEG portions of the macromers confer water solubility properties, and subsequent polymerization results in cell-nonadhesive hydrogels. Glycolic acid oligomers serve as the hydrolyzable fraction of the polymer network, while acrylic end

groups facilitate rapid polymerization and gelation of the macromers.

In preparation for synthesis, glycolide (DuPont) or DL-lactide (Aldrich) was freshly recrystallized from ethyl acetate. PEG oligomers of various molecular weight (Fluka or Polysciences) were dried under vacuum at 110°C prior to use. Acryloyl chloride (Aldrich) was used as received. All other chemicals were of reagent grade and used without further purification.

Macromer synthesis

A 250 ml round bottom flask was flame dried under repeated cycles of vacuum and dry argon. 20 gm of PEG (molecular weight 10,000), 150 ml of xylene and 10 μgm of stannous octoate were charged into the flask. The flask was heated to 60°C under argon to dissolve the PEG and cooled to room temperature. 1.16 gm of glycolide was added to the flask and the reaction mixture was refluxed for 16 hr. The copolymer was separated on cooling and was recovered by filtration. This copolymer was separated on cooling and recovered by filtration. This copolymer (10K PEG-glycolide) was used directly for subsequent reactions. Other polymers were similarly synthesized using DL-lactide or e-caprolactone in place of glycolide and using PEG of different molecular weights.

Synthesis of photosensitive oligomers (macromers):

19 gm of 10K PEG-glycolide copolymer was dissolved in 150 ml methylene chloride and refluxed with 1 ml acryloyl

chloride and 1.2 ml of triethylamine for 12 hr under an argon atmosphere. The solid triethylamine hydrochloride was separated by filtration and the polymer was precipitated by adding the filtrate to a large excess of hexane. The polymer (capped by an acrylate at both ends) was further purified by repeated dissolution and precipitation in methylene chloride and hexane respectively.

Table 12 lists certain macromers synthesized. The degree of polymerization of the glycolide chain extender was kept low so that all polymers have approximately 10 ester groups per chain, or about 5 per chain end. When these polymers are photopolymerized, a crosslinked three-dimensional network is obtained. However, each chain segment in the resulting network needs just one ester bond cleaved at either end to "degrade." These ester cleavages enable the chain to dissolve in the surrounding physiological fluid and thereby be removed from the implant site. The resulting hydrolysis products, PEG and glycolic acid, are water soluble and have very low toxicity.

TABLE 12

Macromers Synthesized

Polymer Code	Mol. Wt. Of Central PEG Chain (daltons)	% Glycolide in Extremities	% ϵ-Caprolactone in Extremities	Calculated Mol. Wt. Of Extremities (daltons)	Appearance
0.4K	400	100	--	580	Viscous liquid
1KG	1000	100	--	300	Viscous liquid
4KG	4000	100	--	232	White solid
10KG	10000	100	--	580	White solid
18.5KG	18500	100	--	1160	Yellow solid
co18.5KGCL	18500	50	--	580	White solid

Due to the presence of only a few units of glycolic acid per oligomeric chain, the solubility properties of the photocrosslinkable prepolymers are principally determined by the central PEG chain. Solubility of the macromers in water and methylene chloride, both of which are solvents for PEG, is not adversely affected as long as the central PEG segment has a molecular weight of 1,000 daltons or more. Solubility data for the prepolymers synthesized is given in Table 13.

Table 13: SOLUBILITY DATA

Solvent	1KG	4KG	10KG	18.5KG	TMP*	
DMSO	–	■	–	■	■	
Acetone	–	■	■	■		–
Methanol	–	■	–	■		–
Water	–	–	–	–	■	
Hexane	■	■	■	■	■	
Methylene Chloride	–	–	–	–		–
Cold Xylene	■	■	■	■	–	
Hot Xylene	–	–	–	–		–
Benzene	■	■	■	■		–

– Soluble

■ Not Soluble

* Trimethylolpropane glycolide triacrylate

PEG chains with different degrees of polymerization of DL-lactide were synthesized to determine the degree of substitution for which water solubility of the macromers can be retained. The results are shown in Table 14. Beyond about 20% substitution of the hydrophilic PEG chain with hydrophobic DL-lactoyl or acrylate terminals leads to the macromers becoming insoluble in water, though they are still soluble in organic solvents such as methylene chloride.

Table 14: Solubility of Macromers

D.P.* of Ethylene Oxide	D.P.* of lactide or glycolide	% extension of PEG chain	Solubility in water
420	4	0.1	soluble
420	10	2.4	soluble
420	20	4.8	soluble
420	40	9.5	soluble
420	80	19	insoluble
23	2	8.7	soluble
23	4	17.4	soluble
23	10	43.5	insoluble
23	40	174	insoluble
5	4	80	insoluble
10	4	40	soluble

* degree of polymerization

Photopolymerization

The macromers can be gelled by photopolymerization using free radical initiators, with the presence of two acrylic double bonds per chain leading to rapid gelation. A 23% w/w solution of various degradable polymers in HEPES buffered saline containing 3 μl of initiator solution (300 mg/ml of 2,2-dimethoxy-2-phenyl-acetophenone in n-vinyl pyrrolidone) was used. 100 μl of the solution was placed on a glass coverslip and irradiated with a low intensity long wavelength UV (LWUV) lamp (Blak-Ray, model 3-100A with flood). The times required for gelation to occur were noted and are given below. These times are typically in the range of 10 seconds. This is very significant because these reactions are carried out in air (UV

initiated photopolymerizations are slow in air as compared to an inert atmosphere) and using a portable, low powered long wave UV (LWUV) emitting source. Oxygen, which often inhibits free radical reactions by forming species which inhibit propagation, did not seem to slow down the polymerization. Such fast polymerizations are particularly useful in applications requiring *in situ* gelations. This rapid gelation is believed to be due to the formation of micelle-like structures between the relatively hydrophobic polymerizable groups on the macromer, thereby increasing the local concentration of the polymerizable species in aqueous solution and increasing polymerization rates.

Visible laser light is also useful for polymerization. Low intensity and short exposure times make visible laser light virtually harmless to living cells since the radiation is not strongly absorbed in the absence of the proper chromophore. Laser light can also be transported using fiber optics and can be focused to a very small area. Such light can be used for rapid polymerization in highly localized regions; gelation times for selected prepolymers are given in Table 15. In each case, 0.2 ml of a 23% w/v photosensitive oligomer solution is mixed with ethyl eosin (10^{-4} M) and triethanol amine (0.01 to 0.1 M) and the solution is irradiated with an argon ion laser (American argon ion laser model 905 emitting at 514 nm) at a power of 0.2-0.5 W/cm^2. The beam is expanded to a diameter of 3 mm and the sample is slowly scanned until gelation occurs.

Table 15: Gelation Times

Polymer	UV polymerization * gelation time (mean ±S.D.) (s)	Laser Polymerization** gelation time (s)
1KG	5.3±4.1	<1
4KG	14.7±0.5	<1
6KG	9.3±0.5	<1
10KG	18.±0.8	<1
10KL	7.7±0.5	<1
18KG	23.3±1.2	<1
20KG	13.3±0.5	<1

* Initiator: 2,2-dimethoxy-2-phenylacetophenone, concentration 900 ppm: 0.2 ml of 23% monomer solution in PBS

** Argon ion laser emitting at 514nm. power 3 W/cm^2: ethyloeosin, triethanol amine initiating system: 0.2 ml of 23% monomer solution in PBS

Biodegradability

Biodegradation of the resulting polymer network is an important criteria in many biomedical applications. Degradation of poly(glycolic acid and poly(DL-lactic acid) has been well documented in the literature. The degradation mainly takes place through the hydrolysis of the ester bond; the reaction is second order and highly pH dependent. The rate constant at pH 10 is 7 times faster than that at pH 7.2.

Such facile biodegradation is surprising because poly(α-hydroxyacidesters) are hydrophobic and highly insoluble in water. Accessibility of the polymer matrix to the aqueous

surrounding is therefore limited. However, because the networks are hydrogels which are swollen with water, all the ester linkages in the network are in constant contact with water with the aqueous surroundings. This results in a uniform bulk degradation rather than a surface degradation of these gels.

Table 16 gives hydrolysis data for some of these networks; times listed are for complete dissolution of 60 mg of gel at pH 7.2 and 9.6. As noted, most of the gels dissolve within 12 hours at pH 9.6. 18.5k gel dissolves within 2.5 hr at pH 9.6 whereas 18.5KCO gel does not dissolve in 3 days, indicating that the lactoyl, glycoloyl, or ε-caprolactoyl ester moiety is responsible for degradation of these networks. It also can be seen that the 18.5KG gel hydrolyzes more rapidly than the 4KG gel. This may be due to the reduced hydrophilicity and higher crosslink density of the latter gel.

Table 16: Hydrolysis Data

Oligomer used for gelation	Time taken to dissolve gel at pH 9.6 (h)	Time taken to dissolve gel at pH 7.2 (days)
4KG	6.2	5.5
10KG	12.25	5.5
18.5KG	2.25	>7
18.5KCL	>5 days	>7
18.5KCO	>5 days	>7

Characterization of macromers

FTIR spectra of the prepolymers were recorded on a DIGILAB model FTS 15/90. The absorption at 1110 cm^{-1} (characteristic C-O-C absorption of PEG) shows the presence of PEG segments. The strong 1760 cm^{-1} absorption shows the presence of glycolic ester. The absence of hydroxyl group absorption around 3400 cm^{-1} and a weak acrylic double bond absorption at 1590 cm^{-1} shows the presence of acrylic double bonds at the end groups.

500 MHz proton and 125 MHz carbon-13 spectra were recorded on a GE 500 instrument. The presence of a very strong peak at 4.9 ppm due to CH_2 methylene from the PEG segment, a peak at 5.09 ppm due to the glycolic ester segment and an acrylic proton singlet at 5.8 ppm can be easily seen from proton NMR. The estimated molecular weight of PEG segment and glycolic acid segment for different copolymers is shown in Table 12. The carbonyl peak at 169.39 ppm from glycolic acid and 36.5 ppm peak from methylene carbons from PEG in carbon-13 NMR are consistent with the reported chemical composition of these copolymers.

Differential scanning calorimetry (Perkin Elmer DSC-7) was used to characterize the oligomers for thermal transitions. The oligomers were heated from -40°C to 200°C at a rate of 20°C/min, presumably causing polymerization. The polymer was then cooled to -40°C at a rate of 60°C/min and again heated to 200°C at a rate of 20°C/min. The first scans of biodegradable

18.5K PEG glycolide tetraacrylate (18.5KG) oligomer were compared to that of the non-degradable 18.5K PEG tetraacrylate (18.5KCO) scan. It was seen that a glass transition appears in the 18.5KG at -2°C while no such transition exists in the 18.5KCO. A small melting peak at 140°C was also evident due to the few glycolic acid mers which can crystallize to a limited extent. The melting peak for PEG is shifted downwards in 18.5KG to 57°C from 60.7°C for 18.5KCO. This is probably due to disturbance of the PEO crystalline structure due to the presence of the glycolic acid linkages. In the third cycle, by which time the oligomers have presumably polymerized, the Tg and Tm transitions for the glycolide segments can no longer be seen, indicating that a crosslinked network has formed and the glycolic acid segments are no longer capable of mobility.

The degree of polymerization (D.P.) of the degradable segments added to the central water soluble PEG chain was determined in several cases using ^{1}H NMR. The experimentally determined D.P. was seen to be in good agreement with the calculated number, as shown by Figure 15. Thus, the ring opening reaction initiated by the PEG hydroxyls proceeds to completion, giving quantitative yields.

Determination of Total Water, Free Water Bound Water

Solutions of various degradable macromers were made as described above. Gels in the shape of discs were made using a mold. 400 μl of solution was used for each disc. The solutions

were irradiated for 2 minutes to ensure thorough gelation. The disc shaped gels were removed and dried under vacuum at 60°C for 2 days. The discs were weighed (W1) and then extracted repeatedly with chloroform for 1 day. The discs were dried again and weighed (W2). The gel fraction was calculated as W2/W1. This data appears in Table 17.

Subsequent to extraction, the discs were allowed to equilibrate with PBS for 6 hours and weighed (W3 after excess water had been carefully swabbed away). The total water content was calculated as (W3-W2) X 100/W3. Differential scanning calorimetry (DSC) was used to determine the amount of free water that was available in the gels. A scan rate of 20°C/min was used and the heat capacity for the endotherm for water melting was measured (H1). The heat capacity of HBS was also measured (H2). The fraction of free water was calculated as H1/H2. The residual water was assumed to be bound due to hydrogen bonding with the PEO segments. The presence of free water in the gels was indicated. This free water can be expected to help proteins and enzymes entrapped in such gels in maintaining their native conformation and reducing deactivation. Thus these gels would appear to be suited for controlled release of biological micromolecules. The data for gel water content is summarized in Table 17.

Table 17: Hydrogel Water content

Polymer Code	% Free Water	% Bound Water	% Total Water	% Gel Content
1KG	68.4	14	82.3±2.6	61.3±5.2
4KG	78.0	9.3	87.3±1.8	56.3±0.9
6KG	74.8	13.4	88.1±3.3	66.5±2.35
10KG	83.7	10.8	94.5±0.5	54.3±0.6
10KL	82.0	9.7	91.7±0.5	63.9±3.7
18.5KG	71.8	22.3	94.0±0.4	47.0±4.9
20KG	79.8	14.8	94.5±0.4	44.5±4.8

Example 34

Use of multifunctional macromers.

30 g of a tetrafunctional water soluble PEG (MW 18,500) (PEG 18.5k) was dried by dissolving the polymer in benzene and distilling off the water benzene azeotrope. In a glove bag, 20 g of PEG 18.5 k, 1.881 g of glycolide and 15 mg of stannous octoate were charged into a 100 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C and 2 hours at 160°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 200 ml of dichloromethane in a 500 ml round bottom flask cooled to 0°C. To this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 h. at 0°C. The triethyl

amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day.

Example 35

Synthesis of a photosensitive macromer containing DL-lactide

PEG (MW) 20,000) (PEG 20k) was dried by dissolving in benzene and distilling off the water benzene azeotrope. In a glove bag, 32.43 g of PEG 20k, 2.335 g of DL-lactide and 15 mg of stannous octoate were charged into a 100 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 200 ml of dichloromethane in a 500 ml round bottom flask cooled to 0°C. To this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 hours at 0°C. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day.

Example 36

Synthesis of a Photosensitive Precursor Containing DL-Lactide and ε-Caprolactone.

PEG (MW 600) (PEG 0.6k) was dried by dissolving in benzene and distilling off the water benzene azeotrope. In a glove bag, 0.973 g of PEG 0.6k, 0.467 g of DL-lactide along with 0.185 g of ε-caprolactone and 15 mg of stannous octoate were charged into a 50 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C and 2 hours at 160°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 50 ml of dichloromethane in a 250 ml round bottom flask cooled to 0°C. to this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 hours at 0°C. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day and was a liquid at room temperature.

Example 37

Selection of dyes for use in photopolymerization

It is possible to initiate photopolymerization with a wide variety of dyes as initiators and a number of electron donors as effective cocatalysts. Table 18 illustrates photopolymerization initiated by several other dyes which have chromophores absorbing at widely different wavelengths. All gelations were carried out using a 23% w/w solution of 18.5KG in HEPES buffered saline. These initiating systems compare favorably with conventional thermal initiating systems, as can also be seen from Table 18. Other photoinitiators that may be particularly useful are 2-methoxy-2-phenyl acetophenone and camphorquinone.

Table 18: Polymerization Initiation of 18.5KG PEG

INITIATOR	LIGHT SOURCE*	TEMPERATURE °C	GEL TIME (SEC)
Eosin Y, 0.00015M; Triethanolamine 0.65M	S1 with UV filter	25	10
Eosin Y, 0.00015M; Triethanolamine 0.65M	S4	25	0.1
Methylene Blue, 0.00024M; p-toluenesulfinic acid, 0.0048M	S3	25	120
2,2-dimethoxy-2-phenyl acetophenone 900 ppm	S2	25	8
Potassium persulfate 0.0168M	-	75	180
Potassium Persulfate 0.0168M; tetramethyl ethylene-diamine 0.039M	-	25	120
Tetramethyl ethylene-diamine 0.039M; Riboflavin 0.00047M	S1 with UV filter	25	300

***LIST OF LIGHT SOURCES USED**

CODE	SOURCE
S1	Mercury lamp, LEITZ WETSLER Type 307-148.002, 100W
S2	Black Ray longwave UV lamp, model B-100A W/FLOOD
S3	MELLES GRIOT He-Ne laser, 10mW output, 1=632 nm
S4	American laser corporation, argon ion laser, model 909BP-15-01001; λ=488 and 514 nm

Numerous other dyes can be used for photopolymerization. These dyes include but are not limited to: Erythrosin, phloxine, rose bengal, thioneine, camphorquinone, ethyl eosin, eosin, methylene blue, and riboflavin. The several possible cocatalysts that can be used include but are not limited to: N-methyl diethanolamine, N,N-dimethyl benzylamine,

triethanol amine, triethylamine, dibenzyl amine, N-benzyl ethanolamine, N-isopropyl benzylamine, and N-vinyl pyrrolidinone.

Example 38

Thermosensitive Biodegradable Gels from N-Isopropyl Acrylamide

Synthesis of low molecular weight polyisopropyl acrylamide

N-isopropyl acrylamide (NIPAAm) was recrystallized from 65:35 hexane benzene mixture. Azobisisobutyronitrile (AIBN) was recrystallized from methanol. 1.5 g of NIPAAm was polymerized using 3 mg of AIBN and 150 mg of mercaptoethanol in 1:1 acetone water mixture (24 hours at 65°C). The viscous liquid after polymerization was purified by dissolving in acetone and precipitating in diethyl ether. Yield 80%.

This hydroxy terminated low molecular weight poly(NIPAAm) was used in chain extension reactions using glycolide and subsequent endcapping reaction using acryloyl chloride as described in other examples.

1 g of modified poly(NIPAAm) based oligomer and 0.2 g 1KL were dissolved in water at 0°C and polymerized at 0°C using 2-2-dimethoxy-2-phenylacetophenone (900 PPM).

Example 39

***In Vitro* Degradation**

The gels were extracted as described in Example 32 to remove the unpolymerized macromer fraction fraction and the gels were then placed in 50 mM HEPES buffered saline (0.9% NaCl), pH 7.4 at 37°C. Duplicate samples were periodically removed, washed with fresh HBS and dried at 100°C for 1 day and weighed to determine mass loss in the gel. The compositions of the various gels used were the same as described in the previous examples. Table 19 shows the extent of degradation of these gels given as percent of mass lost over time. The respective times are given in parenthesis along with the mass loss data.

Table 19: Gel Degradation

1KG	20.1% (1 d), 20.36±0.6 (2d), 21.7± (6d), 28.8±16.6 (10 d) estimated total Degradation time 45 days.
4KG	38.9 (1d), 60.3±4.2 (2d), 78.9 (3d), 99.3±4.7 (6d). Total degradation time 5.5 days.
6KG	18.3±6.8 (1d), 27.4±1.0 (2d), 32.8±11.3 (3d), 104.8±3.2 (5d). total degradation time 4.5 days 10KG 0.6±0.6 (8 hr), 100 (1d). Total degradation time 1 day.
10KL	10.0±4.84 (2d), 6.8±1.7 (3d), 4.5±3.1 (6d), 8.0±0.2 (10d). Total degradation time estimated to be 20 days.
20KG	68.1±4.2 (8hr), 99.7±0.3 (1d). Total degradation time 15 hr.

Example 40

Fibroblast adhesion and spreading

The *in vitro* response of Human foreskin fibroblast (HFF) cells to photopolymerized gels was evaluated through cell culture on polymer networks. 0.2 ml of monomer solution was UV polymerized on an 18 x 18 mm glass coverslips under sterile conditions. HFF cells were seeded on these gels at a cell density of 1.8 x 10^4 cells/sq cm of coverslip area in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum. The gels were incubated for 6 hr at 37°C in a 5% CO_2 environment, at the end of which they were washed twice with phosphate buffered saline (PBS). The adherent cells were fixed using a 2% glutaraldehyde solution in PBS. The gels were examined under a phase contrast microscope at a magnification of 200X, and the number of adherent and spread cells evaluated by examining five fields selected at predetermined locations on the coverslips.

The number of adherent cells is reported in Table 20 along with those for glass control surfaces. Cell adhesion is seen to be dramatically lowered on gel-coated glass.

Table 20: Cell Adhesion

Surface	Attached Cells/cm²
glass	13220±3730
18.5KG	250±240
18.5KCL	1170±1020
18.5KCO	390±150

Typical photographs of these cells on the 18.5KCL gel surfaces and on control glass surfaces are shown in Figures 16A and 16B. It can be easily seen from Table 20 that these gels are highly resistant to cellular growth. Even the 18.5KCL is still less than 10% of the glass. Cells attached to the glass surface show a flattened and well-spread morphology whereas the few cells that are attached to the gel are rounded and loosely attached. This may result from the fact that hydrated PEG chains have a high motility and have been shown to be effective in minimizing protein adsorption. One of the mechanisms by which cell adhesion is mediated is through the interaction of cell surface receptors with adsorbed cell adhesion proteins. Thus the reduction in overall protein adsorption results in minimal cell adhesion protein adsorption and reduced cell adhesion.

Example 41

Release of Protein (Bovine Serum Albumin) from Polymers

1KG was used for this study. This macromer was liquid at room temperature and was used as such. 1 mg of bovine serum albumin (BSA) was added per ml of monomer solution along with 0.9

mg/ml of 2,2-dimethoxy-2-phenyl-acetophenone as initiator. The protein was dissolved in the monomer solution and disc shaped gels were made by exposing 0.2 g of macromer mixture to LWUV for 1 min. Two such discs were placed in a flask containing 20 ml of PBS and incubated at 37°C. Two aliquots of 20 μl each were removed from these flasks periodically and the amount of BSA released was assayed using the Bio-Rad total protein assay. The release profile for BSA is shown in Figure 17A. It can be seen that the release of BSA is relatively steady over more than a month.

Example 42

Enzyme Release Assay

Water solubility of the macromers means gelation can be carried out in a non-toxic environment. This makes these materials suitable for intraoperative uses where *in situ* gelation is needed. Since the precursors are water soluble, the gels can be used as drug delivery vehicles for water soluble drugs, especially macromolecular drugs such as enzymes, which would otherwise be denatured and lose their activity. Release of lysosome and tPA from the polymers was used to illustrate the feasibility of using biodegradable hydrogels for controlled release of biomolecules.

Lysozyme release

The enzyme lysozyme (MW:14,400) is a convenient model for release of a low molecular weight protein from a biodegradable gel. The Biorad total protein assay was used to quantify the enzyme released. The enzyme was dissolved in PBS at a concentration of 20 mg/ml. The monomer PEG-dl-lactic acid-diacrylate was dissolved in PBS to produce a 40% solution. The lysozyme solution was added to the monomer solution to attain a 24% monomer solution. The monomer/lysozyme solution was polymerized under UV in a cylindrical mold, using 30 μl of the initiator 2,2-dimethoxy-2-phenyl-acetophenone in 1-vinyl-2-pyrrolidone (30 mg/ml) as the initiator. The polymer was cut into 10 equal sized pieces and immersed in 10 ml PBS. Samples of the PBS were withdrawn at intervals and assayed for lysozyme released into the PBS. Lysozyme was released from the PEG-DL-lactic acid-diacrylate gel over an 8 day interval, with the maximum rate of release occurring within the first 2 days, as shown by Figure 17B.

Release of recombinant t-PA

Three macromers were used for these studies: 1KL, 4KG, and 18.5KG. The 1KL macromer was liquid at room temperature and was used as such. The second macromer, 4KG, was used as a 75% w/w solution in PBS. The third composition was a mixture of equal parts of 1KL and a 50% w/w solution of 18.5KG. 3.37 mg of tissue plasminogen activator (single chain, recombinant, M.W.

71,000) was added per gram of macromer solution along with 0.9 mg/ml of 2,2 dimethoxy 2 phenyl acetophenone as initiator. The protein was dissolved with the macromer and disc shaped gels were made by exposing 0.2 g of macromer mixture to LWUV for 1 minute. Two such discs were rinsed with PBS, placed in a flask containing 5 ml of PBS and incubated at 37°C. Two aliquots of 100 μl each were removed from these flasks periodically and the amount of active t-PA released was assayed using a chromogenic substrate assay (Kabi-vitrum). The release profiles from the 1K lactide gels, 4K glycolide gels, and the 50/50 1K glycolide/18.5K glycolide are shown in Figures 18A - 18C. Fully active tPA can be released for periods up to at least two months.

By selecting an appropriate formulation, the release rate can be tailored for a particular application. It is also possible to combine formulations with different molecular weights so as to synergistically achieve appropriate attributes in release and mechanical characteristics.

For prevention of postoperative adhesions, in addition to the barrier effect of the gels, the gels can be loaded with a fibrinolytic agent to lyse incipient filmy adhesions which escape the barrier effect. This further enhances the efficacy of biodegradable gels in adhesion prevention.

Example 43

Toxicity of Polymers and Commercial Adhesives

To evaluate the toxicity of *in situ* polymerization of the macromer solutions described herein, as compared to commercial adhesives, 100 μl of 18.5KCO prepolymer solution was placed on the right lobe of a rat liver and gelled by exposing it to LWUV for 15 sec; similarly, a few drops of a n-butyl cyanoacrylate based glue were placed on the left lobe. The liver was excised after a week, fixed in 10% neutral buffered formalin, blocked in paraffin, sectioned and stained using hematoxylin and eosin.

No adverse tissue reaction was evident on the surface of the lobe exposed to the biodegradable gel. No inflammatory reaction to the polymerization process can be seen. The epithelium looks normal, with no foreign body reaction.

In comparison, the lobe exposed to cyanoacrylate glue shows extensive tissue necrosis and scarring with 10-30 cell deep necrotic tissue. Fibrosis is evident in the necrotic portions close to underlying normal tissue.

Example 44

Prevention of Post-Surgical Adhesions with Photopolymerized Biodegradable Polymer

A viscous sterile 23% solution in phosphate buffered saline (8.0 g/l NaCl, 0.201 g/l KCl, 0.611 g/l Na_2HPO_4, 0.191 g/l

KH_2PO_4, pH 7.4) of polyethylene glycol (M.W. 18,500) which has been chain extended on both ends with a short polyglycolide repeat unit (average number of glycolidyl residues: 10 on each end) and which has been subsequently terminated with an acrylate group was prepared. Initiator needed for the crosslinking reaction, 2,2-dimethoxy-2-phenyl acetophenone, was added to the macromer solution to achieve an initiator concentration of 900 ppm. A 30 second exposure to a long wave UV lamp (Blak Ray) is sufficient to cause polymerization.

Animal models evaluated

Animal models evaluated included a rat cecum model and a rabbit uterine horm model. In the rat cecum mode, 6 out of 7 animals treated with the macromer solution showed no adhesions whatsoever, while untreated animals showed consistent dense adhesion formation. In the rabbit uterine horn model, a significant ($p<0.01$) reduction in adhesion formation was seen in the animals treated with the gel. Studies conducted in rats using only the ungelled viscous precursor solution (no LWUV) failed to prevent the formation of adhesions.

Rat cecum model

Twenty-one Sprague Dawley male rats having an average weight of 250 gm were divided into three groups for treatment and two for controls. The abdomen was shaved and prepared with a betadine solution. A midline incision was made under Equithesin anesthesia. The cecum was located and 4 to 5 scrapes were made

on a region about 2 x 1 cm on one side of the cecum, using a 4 x 4 in gauze pad to produce serosal injury and punctate bleeding. The abdominal incisions in these animals were closed using a continuous 4-0 silk suture for the musculoperitoneal layer and 7.5 mm stainless steel staples for the cutaneous layer. A topical antibiotic was applied at the incision site.

The first group consisted of 7 animals serving as controls without treatment, to confirm the validity of the model. The second group served as a control with the application of the precursor but without photopolymerization to form the hydrogel. After induction of the cecal injury, about 0.25 ml of the precursor solution was applied to the injury site using a pipet. The abdominal incision was then closed as above.

The third group served as the gel treatment group and was prepared as the second group except that the precursor film was exposed to a LWUV lamp for 45 seconds to cause gelation. Both the obverse and reverse sides of the cecum were similarly treated with precursor and light. No attempt was made to dry the surface of the tissue, to remove blood, or to irrigate the area prior to treatment.

The animals were sacrificed at the end of two weeks by CO_2 asphyxiation. The incisions were reopened and adhesions were scored for location, extent, and tenacity. The extent of adhesions was reported as a percentage of the traumatized area of the cecum which forms adhesions with adnexal organs or the

peritoneal wall. Tenacity of the adhesions was scored on a scale from 0 to 4: no adhesions - grade 0; tentative transparent adhesions which frequently separate on their own - grade 1; adhesions that give some resistance but can be separated by hand - grade 2; adhesions that require blunt instrument dissection to separate - grade 3; and dense thick adhesions which require sharp instrument dissection in the plane of the adhesion to separate - grade 4.

Rat cecum model results

The control group without treatment shows consistently dense and extensive adhesions. The extent of abraded area covered with adhesions was seen to be 73±21% (mean ± S.D., n=7). The severity of adhesions was grade 3.5±0.4. Most of the adhesions were dense and fibrous, involving the cecum with itself, with the peritoneal wall and with other organs such as the liver, small intestine, and large intestine. Frequently the nesentery was seen to be involved in adhesions. In the control group with the application of precursor solution but without gelation by exposure to the LWUV lamp, the extent of adhesion was 60 ± 24% (n=7), and the severity of adhesions was 3.1 ± 0.4. In the gel treated group, the cecum was seen to be completely free of adhesions in 6 out of 7 animals. In one case, a grade 2 adhesion was seen with the mesentery over 10% of the area and a grade 2.5 adhesion was seen over 15% of the area, bridging the cecum to the sutures on the site of the incision in the

peritoneal wall. The overall adhesion extent for the group was 4%, and the overall severity was 0.32. No evidence of residual gel was visible, the gel presumably having degraded within the prior two weeks. The cecum appeared whitish with a fibrous layer on the surface in the control group, but the tissue appeared healthy and normal in animals treated with the gel.

Rabbit uterine horn model

Eight sexually mature female New Zealand rabbits between 2 and 3 kg in weight were prepared for surgery. A midline incision was made in the lower abdominal region under Rompun, Ketamine, and Acepromazine anesthesia. The uterine horns were located and the vasculature to both horns was systematically cauterized to induce an ischemic injury. One animal was rejected from the study due to immature uterine horns. Seven rabbits were selected for the treatment with only the photopolymerizable hydrogel and two animals were selected for evaluating the combined efficacy of the hydrogel with a fibrinolytic agent, tissue plasminogen activator (tPA). 5 mg of tPA/ml macromer solution was used in the latter case. After cauterization, macromer solutions (0.5 ml) were applied along the horn and allowed to coat the surface where the cauterization injury had been induced. After uniform application of the solution was complete, the horns were exposed to a LWUV lamp for 1 min to induce gelation. The procedure was repeated on the reverse side of the horns. The incisions were then closed using a continuous

2-0 Vicryl (Ethicon) suture for the musculoperitoneal layer and a 0 Vicryl (Ethicon) suture for the cutaneous layer. No prophylactic antibiotics were administered. No postoperative complications or infections were observed. Five animals were used in the control group. The ischemic injury was made as described and the incision was closed without the application of the precursor; all techniques were identical between the treatment group and the control group.

Controls were used where the same animal model was subjected to surgery without application of the macromer; all surgical techniques were identical between the treatment group and the historical controls.

The rabbits were reoperated under Ketamine anesthesia at the end of two weeks to evaluate adhesion formation; they were sacrificed by introcardiac KCl injection. Adhesion formation was evaluated for extent and tenacity. Extent of adhesion formation was evaluated by measuring the length of the uterine horn that formed adhesions with itself or with the peritoneal wall or other organs. Tenacity of adhesion was classified as either filmy or fibrous. Filmy adhesions were usually transparent, less strong, and could be freed by hand. The fibrous adhesions were dense, whitish, and usually required sharp instrument dissection to be freed. In cases where only a single filmy adhesion band was evident, a score of 5% was assigned.

Typical samples of the horn were excised for histology and were fixed in a 10% neutral buffered formalin solution. Paraffin sections of the samples were stained using hematoxylin and eosin.

Rabbit uterine horn model results

The adhesion score is the % of affected area occupied by the adhesions, with grading of each as being filmy or fibrous. Distorted horn anatomies were observed in control animals. The mean score in the control group was 50 ± 15% of the affected area of the horn being occupied by adhesions with 10% of these being filmy and 90% fibrous. Distorted horn anatomies were observed, as can be seen from Figure 19A which presents a superior view of the uterine horn in an animal used as a control, which showed adhesions over 66% of the horn surface. The group of animals treated only with the photopolymerized macromer showed an adhesion score of 13±11.4% (n=10). Of these, 4 animals showed less than 5% adhesions with only an occasional filmy band visible.

The animals treated with photopolymerized gel containing tPA showed further improved results over the "gel only" animals. One animals showed a filmy band on both the right and left horn. They were assigned a score of 5% with a total score of 10%. The other animal did not show any adhesions at all. Thus the total score for these animals was 5±5%.

Figure 19B shows normal horn anatomy in a typical horn which has undergone gel treatment. Adhesions are filmy in all cases and no dense bands are seen. No traces of the remaining gel could be observed. Typical samples of horns showing filmy adhesions showed some fibrous tissue with a 6-15 cell thick layer of fibroblasts showing some collagen fibrils but no formation of dense collagen fibers. The horns showing no adhesions occasionally showed a 1-4 cell thick layer of fibroblasts, but mostly a normal epithelium with no evidence of inflammatory cells.

This same procedure was slightly modified as described below as a better mode of using the polymers to prevent postoperative adhesions using the rat uterine horn model.

Female rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally), and a midline laparotomy was performed. The uterine horns were exposed, and the vasculature in the arcade feeding the horns was systematically cauterized using bipolar cautery; the most proximal and most distal large vessel on each horn were not cauterized. Following this, the antimesenteric surface of each horn was cauterized at two 1 mm diameter spots on each horn, each separated by a 2 cm distance, the pair centered along the length of each horn. Following injury, 0.5 ml of macromer solution was applied per horn and was gelled by exposure to long wavelength ultraviolet light (365 nm, approximately 20 mW/cm^2) for 15 sec per surface on the front side

and on the back side each. The uterus was replaced in the peritoneal cavity, and the musculoperitoneal and skin layers were closed.

The macromer consisted of a PEG chain of MW 8,000 daltons, extended on both sides with a lactic acid oligomer of an average degree of polymerization of 5 lactidyl groups, and further acrylated nominally at both ends by reaction with acryloyl chloride. In one batch, Batch A, the degree of acrylation was determined by NMR to be approximately 75%, and in another, Batch B, it was determined to be greater than approximately 95%. The macromer was dissolved in saline at a specified concentration, and the initiation system used was 2,2-dimethoxy-2-phenyl acetophenone from a stock solution in N-vinyl pyrrolidinone, the final concentration of 2,2-dimethoxy-2-phenyl acetophenone being 900 ppm and the final concentration of N-vinyl pyrrolidinone being 0.15%.

In one set of experiments, macromer from Batch A was applied in varying concentrations, and adhesions were scored at 7 days postoperatively. Scoring was performed by two means. The length of the horns involved in adhesions was measured with a ruler, and the fraction of the total length was calculated. The nature of the adhesions was also scored on a subjective scale, 0 being no adhesions, 1 being filmy adhesions that are easily separated by hand, and 2 being dense adhesions that can only be separated by sharp instrument dissection. Furthermore, one of

the samples contained tissue-plasminogen activator (t-PA), which is known to reduce adhesions, at a concentration of 0.5 mg/ml (0.5%) macromer solution. The results are shown in Table 21 for macromer batch A and batch B.

In a third set of experiments, adhesions were formed in female rats as described above, and the adhesions were surgically lysed 7 days after the initial surgery. The extent and grade of adhesions was scored during lysis. The animals were divided into two groups, and one group was treated with macromer from Batch B at a concentration of 10%. The results are shown in Table 21 as batch B, 10%.

Table 21: Reduction of Adhesions with Polymer.

Concentration	Extent of adhesions % (S.D.)	Grade of adhesions (0-2)	Number of Animals	macromer
Polymer A				
15%	24.6 (3.1)	1.1 (0.1)	7	
20%	33.6 (9.8)	1.2 (0.3)	7	
25%	37.5 (11.1)	1.2 (0.1)	7	
30%	54.2 (12.0)	1.6 (0.4)	6	
20% + t-PA	18.3 (6.4)	1.1 (0.1)	6	
Control (saline)	72.6 (18.7)	1.5 (0.2)	7	
Polymer B				
5%	22.1 (4.2)	1.2 (0.1)	7	
10%	10.0 (5.1)	1.0(0)	7	
15%	17.8 (5.7)	1.0 (0)	7	
20%	26.3 (11.4)	1.4 (0.2)	7	
Control (saline)	75.9 (4.4)	1.8 (0.3)	7	
Polymer B, 10%				
Scoring performed at:	group that became:			
time of lysis	Controls	85.9 (9.7)	1.8 (0.1)	7
Time of lysis	Treatment	79.4 (6.8)	1.7 (0.2)	7
7 days post-lysis	Controls	78.8 (11.3)	1.8 (0.1)	7
7 days post-lysis	Treatment	28.2 (5.1)	1.0 (0)	7

The above results illustrate that the photopolymerized macromer can reduce or prevent post operative adhesions in both primary adhesions and adhesiolysis models, and moreover that the gel can be used to locally release a drug to exert a combined beneficial effect.

Example 45

Nerve anastomosis

The sciatic nerve of a rat was aseptically severed using a scalpel and allowed to pull apart. The two ends of the nerve were reopposed using sterile forceps, and a 50% solution in buffer of polymer 1KL, a macromer made from PEG 1K with lactide chain extension and acrylate termination, with 0.1% 2,2-dimethoxy-2-phenoxy acetophenone was applied to the nerve stumps. The affected area was illuminated with a 100 W LWUV lamp for 60 seconds, and an adhesive bond was observed to form between the proximal and distal nerve stumps.

To ensure the biocompatibility of the applied material with the nerve tissue, the same solution of macromer was applied to nonsevered rat sciatic nerves, and the area of the incision was closed using standard small animal surgical technique. The area was reopened at 1 hour or 24 hour postoperatively, and the affected area of the nerve was removed en block and prepared for transmission electron microscopy. No morphological differences were observable between the treated nerves at either time point as compared to control rat sciatic nerves that were otherwise nonmanipulated, even though they had been traumatized and manipulated.

Example 46

Evaluation of PEG Based Degradable Gels as Tissue Adhesives

Abdominal muscle flaps from female New Zealand white rabbits were excised and cut into strips 1 cm X 5 cm. The flaps were approximately 0.5 to 0.8 cm thick. A lap joint, 1 cm X 1 cm, was made using two such flaps. Two different compositions, 0.6KL and 1 KL, were evaluated on these tissues. Both these compositions were viscous liquids and were used without further dilution. 125 μl of ethyl eosin solution in N-vinyl pyrrolidone (20 mg/ml) along with 50 μl of triethanolamine was added to each ml of the adhesive solution. 100 μl of adhesive solution was applied to each of the overlapping flaps. The lap joint was then irradiated by scanning with a 2 W argon ion laser for 30 sec from each side. The strength of the resulting joints was evaluated by measuring the force required to shear the lap joint. One end of the lap joint was clamped and an increasing load was applied to the other end, while holding the joint was clamped and an increasing load was applied to the other end, while holding the joint horizontally until it failed. Four joints were tested for each composition. The 1KL joints had a strength of 6.6±1.0 KPa (mean ±S.D.), while the 0.6KL joints had a strength of 11.4±2.9KPa. It is significant to note that it was possible to achieve photopolymerization and reasonable joint strength despite the 6-8 mm thickness of tissue. A spectrophotometric estimate

using 514 nm light showed less than 1% transmission through such muscle tissue.

Example 47

Coupling of Photopolymerizable Groups to Proteins (Albumin)

PEG (M.W. 2,000) monoacrylate (5g) was dissolved in 20 ml dichloromethane. Triethyl amine (0.523 g) and 2,2,2-trifluoroethanesulfonyl chloride (tresyl chloride) (0.017 g) were added and the reaction was allowed to proceed for 3 hours at 0°C under nitrogen atmosphere. The reaction mixture was then filtered and the dichloromethane evaporated to dryness. The residue was redissolved in a small amount of dichloromethane and precipitated in diethyl ether. The polymer was then filtered and dried under vacuum for 10 hours and used directly in the subsequent reaction with albumin.

1 g of bovine serum albumin was dissolved in 200 ml of sodium bicarbonate buffer at pH 9. Tresyl activated PEG monoacrylate (5 g) was added and the reaction was stirred for 24 hours at 25°C. Albumin was separated by pouring the reaction mixture into acetone. It was further purified by dialysis using a 15,000 daltons cutoff dialysis membrane. A 10% w/v solution of the PEG acrylated albumin could be photopolymerized with long wave UV radiation using 0.9 mg/ml of 2,2 dimethoxy 2 phenylacetophenone as the initiator. In this gel the degradable segment is the protein albumin.

Example 48

Modification of Polysaccharides (Hyaluronic Acid)

In a dry 250 ml round bottom flask, 10 grams of PEG 400 monomethacrylate was dissolved in 100 ml dry dioxane, to which 4.053 g of carbonyl diimidazole (CDI) was slowly introduced under nitrogen atmosphere and the flask was heated to 50°C for 6 h. Thereafter the solvent was evaporated under vacuum and the CDI activated PEG monomer was purified by dissolving in dichloromethane and precipitating in ether twice.

1 g of hyaluronic acid, 5 g of CDI activated PEG 400 monoacrylate were dissolved in 200 ml sodium borate buffer (pH 8.5) and the solution was stirred for 24 hours. It was then dialyzed using a 15,000 dalton cutoff dialysis membrane to remove unreacted PEG. A 10% w/v solution of the acrylated hyaluronic acid was photopolymerized with long wave UV radiation, using 0.9 mg/ml of 2,2-dimethoxy-2-phenylacetophenone as the initiator. In this gel, the degradable region is hyaluronic acid.

Example 49

PEG Chain Extended with Polyorthocarbonates and Capped With Urethane Methacrylate

3, 9-bis(methylene) 2,4,8,10-tetraoxaspiro [5,5] undecane (1g) and polyethylene glycol (molecular weight, 1,000, 7.059 g) were weighed into a 250 ml Schlenk tube under dry nitrogen atmosphere in a glove bag. 50 ml of dry tetrahydrofuran

was introduced under nitrogen atmosphere and reaction mixture was stirred for 6 hours at 50°C. This is a typical step growth reaction with a disturbed stoichiometry, resulting in low molecular weight poloyorthocarbonate with terminal hydroxy groups. The oligomer was separated by precipitating in hexane and dried under vacuum. 5 g of oligomer was redissolved in dry THF to which 20 μl of dibutyltindilaurate and 2 ml of 2-isocyanatoethyl methacrylate were slowly introduced and temperature was raised to 50°C. It was held there for 6 hours and cooled. The product was separated by precipitation in hexane. In this gel, the degradable region is a polyorthocarbonate.

Example 50

Microencapsulation of Animal Cells

A 23% w/w solution of 18.5KG in HEPES buffered saline (5 ml) was used to resuspend 10^6 CEM-SS cells. Ethyl eosin (10^{-4} M) was used as a solution in N-vinyl pyrrolidone as the initiator and triethanolamine (0.01 M) was used as the coinitiator. The solution was then exposed through a coextrusion apparatus to an argon ion laser (514 nm, 2 Watts). The coextrusion apparatus had mineral oil as the fluid flowing annularly (flow rate 4 ml/min) around an extruding stream of the precursor cell suspension (flow rate 0.5 ml/min). The microdriplets gelled rapidly on being exposed to the laser light and were collected in a container

containing PBS. The oil separated from the aqueous phase and the microspheres could be collected in the PBS below. The microspheres formed were thoroughly washed with PBS buffer to remove unreacted monomer and residual initiator. The size and shape of microspheres was dependent on extrusion rate and extruding capillary diameter (18 Ga to 25 Ga). The polymerization times were dependent on initiator concentration (ethyl eosin 5 μM to 0.5 mM, vinyl pyrrolidone (0.001% to 0.1%), and triethanolamine (5 mM to 0.1 M), laser power (120 mW to 2W), and monomer concentration (>10%w/v). Spheres prepared using this method had a diameter from 500 μm to 1,200 μm. The polymerizations were carried out at physiological pH in the presence of air. This is significant since radical polymerizations may be affected by the presence of oxygen. Cell viability subsequent to encapsulation was checked by trypan blue exclusion assay and the encapsulated cells were found to be more than 95% viable after encapsulation.

Example 51

Various Formulations for the Prevention of Post Operative Adhesions

The utility of PEG-oligo(α-hydroxy acid) diacrylates and tetraacrylates to prevent postoperative adhesions was evaluated in the rabbit uterine horn model as described above. The following polymers were synthesized, as described above: PEG

6K lactide diacrylate (6KL), PEG 10K lactide diacrylate (10KL). PEG 18.5K lactide (18.5KL), PEG 20K lactide (20KL). Solutions with 24% polymer in PBS with 900 ppm 2,2-dimethoxy-2-phenyl acetophenone, were prepared as described above. The solutions were applied to the uterine horn after cautery of the vascular arcade and illuminated with a 365 nm LWUV lamp, as described above. In one formulation, 18.5KL, 5 mg t-PA was mixed into the solution before application. Controls consisted of animals manipulated and cauterized but not treated with macromer solution. Measurement was performed on the 14th ± 1 day. Extent of adhesion was estimated from the fraction of the horn that was involved in adhesions, and the tenacity of adhesions was scored as 0, no adhesions; 1, filmy adhesions that offer no resistance to dissection; 2, fibrous adhesions that are dissectable by hand; 3, fibrous adhesions that are dissectable by blunt instruments; and 4, fibrous adhesions that are dissectable by sharp instruments. The results were as follows, where the extent of adhesions and the tenacity of the adhesions are shown.

Table 22: Efficacy of Polymer in Preventing Adhesions.

Formulation	Number of animals	Extent, %, ± S.D.	Tenacity, 0-4 ± S.D.
6KL	7	0.9 ± 1.7	0.9 ± 0.7
10KL	7	0 ± 0	0 ± 0
20KL	6	4.4 ± 5.0	0.9 ± 0.7
18.5KL	7	8.9 ± 13.1	1.6 ± 1.3
t-PA Control	7	35 ± 22	3.3 ± 0.6

Example 52

Polymerization of Ultrathin Layers of Polymer on the Surface of Blood Vessels to Reduce Thrombosis After Vessel Injury

Blood vessels were harvested from rats and were rinsed free of blood. The endothelium of the vessel were removed by inserting a wooden dowel and rotating the vessel over the dowel. One vessel was used as a control, and was exposed to flowing blood as described below without further modification. Another vessel was treated first by exposure to eosin Y at 1 mM in saline, then rinsed in HEPES buffered saline, then filled with a solution of PEG-MA, PEG 10K with acrylate end-capped oligomers of DL lactide, containing triethanolamine (TEA) (100 mM) and N-vinylpyrrolidone (VP) (0.15%) and then illuminated by exposure to an argon ion laser at 0.5 W/cm2 for 15 sec. The nonpolymerized prepolymer mixture in the lumen of the vessel was rinsed away with saline. Human blood was collected from the antecubital vein and was anticoagulated with heparin at 2 units/ml. This blood

was perfused through each vessel by a syringe pump at a flow rate corresponding to a wall shear rate of approximately 200/s for 7 min. The vessel was then superficially rinsed in saline and fixed in formaldehyde.

The treated vessel did not appear colored or different in color after perfusion compared to its color before perfusion, while the untreated control vessel appeared blood red. Thin segments of each vessel were cut from each vessel, were mounted on end, and were examined by environmental scanning electron microscopy (ESEM). ESEM is performed on hydrated samples in relatively low vacuum. This permits the visualization of the polymer film coating in the swollen and wet state. This is important to obtain measurements that may be readily interpreted, since the polymer film is approximately 95% water. A high degree of thrombosis was readily observed in the control vessel. The lumen of this vessel was narrowed to less than one-third its diameter pre-perfusion by the accumulation of thrombus, as shown in Figure 20A. By contrast, no thrombus could be observed in the lumen of the treated vessel, as shown in Figure 20B. A higher magnification of the vessel wall demonstrated no adherent thrombus. A still higher magnification shows a white structure which is the polymer film, which is different in contrast from the tissue due to differential charging under the electron beam of the ESEM. The film may be seen to be precisely conformed to the shape of the vessel and be approximately 5 - 8 μm thick.

The region of polymerization was restricted to the neighborhood of the blood vessel wall surface. The photosensitive dye was adsorbed to the vessel wall. Unbound dye was rinsed away. The entire lumen was filled with prepolymer, but upon illumination the gel formation was restricted to the vessel wall where the dye and the prepolymer meet. This interfacial polymerization process can be conducted to produce surface adherent layers that vary in thickness from less than 7 μm to more than 500 μm.

The above procedure was performed in 8 control rat arteries, and 8 treated arteries, with equivalent light microscopic histological results as described above. As demonstrated by this study, PEG prepolymers can be polymerized upon the lumenal surface of blood vessels. The immediate effect of this modification is to reduce the thrombogenicity of an injured blood vessel surface. This has clear utility in improving the outcome of balloon angioplasty by reducing the thrombogenicity of the vessel and lesion injured by balloon dilation. Another effect of this modification is to be reduce smooth muscle cell hyperplasia. This may be expected for two reasons. First, platelets contain a potent growth factor, platelet-derived growth factor (PDGF), thought to be involved in post-angioplasty hyperplasia. The interruption of the delivery of PDGF itself poses a pharmacological intervention, in that a "drug" that would have been delivered by the platelets would be

prevented from being delivered. Thrombosis results in the generation of thrombin, which is a known smooth muscle cell mitogen. The interruption of thrombin generation and delivery to the vessel wall also poses a pharmacological intervention. There are other growth factors soluble in plasma which are known to be smooth muscle cell mitogens. The interruption of thrombin generation and delivery to the vessel wall also poses a pharmacological intervention. Moreover, there are other growth factors soluble in plasma which are known to be smooth muscle cell mitogens. The gel layer is known to present a permselective barrier on the surface of the tissue, and thus the gel layer may reasonably be expected to reduce hyperplasia after angioplasty. The inhibition of thrombosis upon the vessel wall may also reduce the incidence of abrupt reclosure and vasospasm, both of which occur sometimes following vascular intervention.

Example 53

Interfacial Polymerization of Macromers Inside Blood Vessels to Prevent Thrombosis

Macromer solutions were polymerized interfacially within previously injured blood vessels *in vivo* to prevent thrombosis. The carotid artery was exposed, and a polyethylene tube (PE-10) was used to cannulate the exterior carotid artery. The artery was clamped with fine arterial clamps proximal to the interior/exterior carotid artery bifurcation and approximately 2

cm distal to the bifurcation. A 1 ml tuberculin syringe was used to rinse the blood from the lumen of the isolated zone by filling and emptying the vessel zone. The vessel was injured by crushing using a hemostat. The isolated zone was filled with a 10 mM solution of eosin Y for 2 minutes, after which it was rinsed and filled with a 20% solution of a macromer in saline with 0.1 mM triethanolamine and 0.15% N-vinyl pyrrolidinone. The macromer consisted of a PEG chain of MW 8,000 daltons, extended on both sides with a lactic acid oligomer of an average degree of polymerization of 5 lactidyl groups, and further acrylated nominally at both ends by reaction with acryloyl chloride. The vessel was illuminated transmurally using an argon ion laser (514 nm) at an intensity of approximately 1 mW/cm^2 for 5 seconds. Following this, the cannula was removed from the exterior carotid artery and the artery was ligated at the bifurcation. The arterial clamps were removed to permit the resumption of blood flow. Perfusion was allowed for 20 minutes, following which the vessel were again isolated, removed from the body, gently rinsed, fixed, and prepared for light microscopic histological analysis. Using the naked eye, the crushed segments in control animals, which lacked illumination, were red, indicating internal thrombus with entrapped red blood cells. By contrast, no redness was observed at the site of the crush injury in the treated vessels. Histology showed extensive thrombus, fibrin, and entrapped red blood cells in the non-treated vessels. By contrast, no thrombus

or fibrin or entrapped red blood cells were observed in the treated vessels. The procedure was conducted in four control animals and three treated animals.

This example demonstrates that the polymerization can be carried out *in situ* in the living animal, that the polymer coating remains adherent to the vessel wall during arterial blood flow, and that the polymer coating can prevent thrombosis *in vivo* in non-anticoagulated animals. This approach to treatment has clear benefits in preventing abrupt reclosure, vasospasm, and restenosis after intravascular interventional procedures. Moreover, it is more generally applicable to other intraluminal and open-surface organs to be treated.

9. The crosslinked biocompatible material of claim 8, wherein the biologically active materials are living cells selected from islet of Langerhans, dopamine secreting cells, crythropoietin secreting cells, nerve growth factor secreting cells, parathyroid cells, or norepinephrine/-metacephalin secreting cells.

10. The crosslinked biocompatible material of claim 8, wherein the biologically active material is a drug

11. A crosslinked biocompatible material comprising: at least one ionically crosslinked component; and at least one covalently crosslinked component is derived from a polyalkylene oxide.

12. The crosslinked biocompatible material of claim 11, wherein the covalently crosslinked component is polyethylene glycol diacrylate.

13. The crosslinked biocompatible material of claim 11, further comprising a biologic encapsulated by the material.

14. The crosslinked biocompatible material of claim 13, wherein the material is effective to provide immunoprotection for the biologic in a physiological environment.

15. The crosslinked biocompatible material of claim 14, wherein the material provides immunoprotection of the biologic when xenotransplanted.